PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/86, 5/10, A61K 39/275	A1	11) International Publication Number: WO 97/37031
		43) International Publication Date: 9 October 1997 (09.10.97)
(21) International Application Number: PCT/NZ	′97/000	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE,
(22) International Filing Date: 27 March 1997 (GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,	
(30) Priority Data: 286284 29 March 1996 (29.03.96)	N	LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI
(71) Applicant (for all designated States except US): UNIV OF OTAGO [NZ/NZ]; Leith Street, Dunedin (NZ)		patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(72) Inventors; and (75) Inventors/Applicants (for US only): ROBINSON, John [NZ/AU]; 12 Maranoa Street, Kaleen, Canbe 2617 (AU). LYTTLE, David, James [NZ/NZ]; 18 Road, Highcliff R.D. 2, Dunedin (NZ).	erra, AC	Published With international search report.
(74) Agents: BENNETT, Michael, R. et al.; A.J. Park Huddart Parker Building, 6th floor, Post Office Squ Box 949, Wellington 6015 (NZ).	: & So: iare, P.0	

(54) Title: PARAPOXVIRUS VECTORS

(57) Abstract

The invention is directed to parapoxvirus vectors. Specifically provided are orf virus vectors containing exogenous DNA. The exogenous DNA may encode a heterologous peptide or polypeptide of which expression is desired, or may encode an antigen capable of inducing an immune response. The capacity to express antigens make these vectors suitable for use in vaccines.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	zw	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

- 1 -

PARAPOXVIRUS VECTORS

TECHNICAL FIELD

5

This invention relates to parapoxvirus vectors, methods for their construction, and uses thereof.

BACKGROUND OF THE INVENTION

10

15

Poxviruses are large DNA viruses which replicate within the cytoplasm of infected cells. A number of members of the poxvirus family have been used to express foreign genes. These members include vaccinia virus and avipox virus. Such viruses have the potential to deliver vaccine antigens to a variety of animal species. However, the use of modified vaccinia virus and avipox viruses are subject to a number of drawbacks.

Vaccinia virus has a wide host range in mammals. Accordingly, there is a significant risk of cross-species infection and consequent spread of disease from one species to another. This represents a significant disadvantage for any vector being used in the environment.

20

A further disadvantage is that vaccinia virus especially, has been shown to cause a febrile response and scarring in humans and occasionally, serious disease in an infected animal.

Avipoxviruses are more variable in their host range specificity, and while they will not generally propagate in mammals, they will often undergo an abortive infection sufficient to induce an immune response to at least some foreign genes if they are incorporated into the genome of the avipoxvirus and are expressed under control of the appropriate promoter.

Also the first infection with a vaccinia virus vector will induce an immunity to the vector such that it may limit the potential of a subsequent infection with the vector to deliver a full dose of antigen.

In the agricultural context, a major limitation to livestock production is the control of parasitic diseases. As drench resistance builds up in farmed animal populations, and consumer resistance to the use of chemical agents in livestock production also increases, there is a need for alternative means of disease control. Use of cheap, safe and effective

vaccines using parapox virus vectors to deliver antigens to the host is one alternative solution which addresses these problems.

The concept of parapox virus vectors and more particularly orf virus vectors is disclosed generally by Robinson, A.J. and Lyttle, D.J. "Parapoxviruses: their biology and potential as recombinant vaccines" in Recombinant Poxviruses, Chapter 9, 306-317 eds M.Binns and G. Smith CRC Press, (1992), Boca Raton. However, there is no teaching in the reference of suitable gene insertion sites or sequences coding therefor which would allow orf virus to be used as a vector.

10

It is therefore an object of the present invention to provide a virus vector which goes some way toward overcoming the disadvantages outlined above in relation to existing poxvirus vectors or which at least provides the public with a useful choice.

15 **SUMMARY OF THE INVENTION**

Accordingly, in one aspect, the present invention provides a parapoxvirus vector comprising a parapox virus containing exogenous DNA.

20 Preferably, the parapox virus is orf virus.

Desirably, the exogenous DNA encodes at least one gene product, and most usefully this product will be an antigen capable of inducing an immune response.

In addition, the exogenous DNA preferably further encodes at least one gene product which is a biological effector molecule, most usefully a cytokine which is capable of acting as an immunological adjuvant.

In addition, the exogenous DNA also preferably encodes a peptide moiety expressed as a hybrid or chimeric protein with a native virus protein.

Also within the scope of the invention are fragments or variants of the vector having equivalent immunological activity.

35 It is desirable that the exogenous DNA be incorporated in a non-essential region of the virus genome.

The exogenous DNA is preferably under the control of a poxvirus promoter, and conveniently an orf virus promoter.

In a further aspect, the present invention provides a method for the production of parapoxvirus vectors, replicable transfer vectors for use in the method of the invention and hosts transformed with these vectors.

In a further aspect the invention consists in a vaccine which includes a parapoxvirus vector defined above in combination with a pharmaceutically acceptable carrier and optionally or alternatively, an adjuvant therefor.

In a still further aspect the present invention relates to the use of parapoxvirus vectors to prepare heterologous polypeptides in eukaryotic cells comprising infecting cells with the parapoxvirus vector and isolating the heterologous polypeptide once expressed.

Although the invention is broadly as described above, it will be appreciated by those persons skilled in the art that the invention is not limited to the foregoing but also includes embodiments of which the following gives examples. In particular, certain aspects of the invention will be more clearly understood by having reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

15

20

25

30

35

Figure 1 represents a map of the genomes of the orf virus strains NZ-2, NZ-7 and NZ-10 showing cleavage sites for the restriction endonuclease KpnI. The genomes are double stranded DNA molecules and are represented as horizontal lines. The positions of the endonuclease cleavage sites on each genome relative to the ends of the genome are represented by vertical lines. Individual genome fragments that would be generated by digestion with the endonuclease are designated with letters of the alphabet.

Figure 2 represents a nucleotide sequence of a region of the *Kpn*l E fragment of the orf virus strain NZ-2 genome. The sequence underlined with a dashed line contains potential insertion sites. The sequence underlined with colons represents that portion of a vascular endothelial growth factor like gene that contains potential insertion sites.

Figure 3 represents a nucleotide sequence of a region of the *Kpn*l D fragment of the orf virus strain NZ-7 genome in Figure 1. The sequences underlined with a dashed line represent sites for the insertion of foreign genes. The sequence underlined with colons

- 4 -

represents that portion of a vascular endothelial growth factor-like gene that contains potential insertion sites.

Figure 4 represents a map of the genome of the orf virus strain NZ-2 showing cleavage sites for the restriction endonuclease *Hind*III. The genome is a double stranded DNA molecule and is here represented as a horizontal line. The positions of the endonuclease cleavage sites on the genome relative to the ends of the genome are represented by vertical lines. Individual genome fragments that would be generated by digestion with the endonuclease are designated with letters of the alphabet. The region comprising part of fragment F, all of fragments J and I and part of fragment E for which the DNA sequence has been determined is shown. Open reading frames encoding putative genes are shown. The open reading frames encoding the putative genes (H)I1L and (H)I2L contain potential insertion sites. In addition the intergenic regions between rpo132 and (H)I1L, (H)I1L and (H)I2L, (H)I2L and (H)E1L and (H)E2L represent potential insertion sites.

15

10

Figure 5 represents the nucleotide sequence of the open reading frames depicted in Figure 4. The genes (H)I1L, and (H)I2L which contain potential insertion sites are underlined with colons. Potential insertion sites within intergenic regions are underlined with a dotted line. Putative promoter sequences are marked by asterisks.

20

Figure 6 represents a map of the genome of the orf virus strain NZ-2 showing cleavage sites for the restriction endonuclease BamHI The genome is a double stranded DNA molecule and is here represented as a horizontal line. The positions of the endonuclease cleavage sites on the genome relative to the ends of the genome are represented by vertical lines. Individual genome fragments that would be generated by digestion with the endonuclease are designated with letters of the alphabet. The region comprising fragment BamHI F and part of BamHI C for which the DNA sequence has been determined is shown. Open reading frames encoding DNA topoisomerase (F4R) and the putative genes F1L, F2L, F3R and C1L are shown as unfilled arrows.

30

Figure 7 represents a nucleotide sequence of the BamHI F fragment and part of the BamHI C fragment of the orf virus strain NZ-2 genome shown in Figure 6. The sequences underlined with a dashed line represent potential insertion sites. The putative promoter sequences PF1L, PF2L, PF3R, PF4R and PC1R are marked by asterisks.

35

Figure 8 represents a map of the genome of orf virus strain NZ-2 showing cleavage sites for the restriction endonuclease *BamHI*. The genome is a double stranded DNA molecule and is here represented as a horizontal line. The positions of the endonuclease cleavage

- 5 -

sites on the genome relative to the ends of the genome are represented by vertical lines. Individual genome fragments that would be generated by digestion with the endonuclease are designated with letters of the alphabet. The region comprising fragments *Bam*HI H, *Bam*HI E, *Bam*HI G and part of *Bam*HI B for which the DNA sequence has been determined is shown. Open reading frames encoding putative genes are shown as unfilled arrows. The position of a 3.3 kilobase pair deletion encompassing open reading frames E2L, E3L and G1L is shown.

Figure 9 represents a nucleotide sequence of a region of the BamHI E fragment and BamHI G fragment of the orf virus strain NZ-2 genome shown in Figure 8. Potential insertion sites underlined by colons are present in the region which encodes for the putative genes E2L, E3L and G1L. Potential insertion sites within intergenic regions are underlined with a dotted line. Putative promoter sequences are marked by asterisks. The region located between the ITR junction and the marked endpoint of deletion is absent in a variant strain derived from NZ-2.

15

20

25

30

35

Figure 10 represents nucleotide sequences from the orf virus genome strain NZ-2 that act as transcriptional promoters. Early and late promoter sequences are indicated. For each sequence the left hand end is the 5' end.

Figure 11 is a diagram representing the steps in the construction of the plasmid pSP-PFlac.

Figure 12 is a diagram representing the steps in the construction of the plasmid pSP-SFPgpt32.

Figure 13 is a diagram representing the steps in the construction of the plasmid pFS-gpt.

Figure 14 is a diagram representing the steps in the construction of the plasmids pVU-DL104 and pVU-DL106.

Figure 15 is a diagram representing the steps in the construction of the plasmids ptov2 and ptov3.

Figure 16 is a diagram representing the steps in the construction of the plasmid ptov6.

Figure 17 is a diagram representing the steps in the construction of the plasmid ptov8.

Figure 18 is a diagram representing the steps in the construction of the plasmids pVU-DL45W and pVU-DL45Wl.

Figure 19 is a diagram representing the steps in the construction of the plasmids pVU-5 DL45Wlac and pVU-DL45Wlac.

Figure 20 outlines a strategy for the generation of recombinant orf virus.

Figure 21A provides the nucleic acid sequence for the primers zxs-1, zxs-2, zxs-3 and zxs-10 4 used for the amplification of orf virus sequences used to create the transfer vector pTvec50.

Figure 21B provides the nucleic acid sequence for the modified intergenic region between the RNA polymerase subunit gene, rpo 132, and (H)I1L in pTvec50, showing new created restriction sites for the restriction enzymes Apol, Nsil, Ncol and EcoRI. The priming sites on the original OV sequence for the zxs-3 primer are marked by asterisks, the newly created transcriptional termination signal (TTTTTAT) is shown in bold type.

Figure 22 is a diagram representing the steps in the construction of the plasmids pTvec1 and pTvec-50.

Figure 23 is a diagram representing the steps in the construction of the transfer vectors pTvec50lac-1 and pTvec50lac-2.

In a first aspect the present invention provides a parapoxvirus vector comprising a parapox virus containing exogenous DNA. Preferably, the parapoxvirus is an orf virus. Orf virus has a relatively narrow host range being generally confined to sheep, goats, monkeys and man. The narrow host range avoids the disadvantage associated with the use of vaccinia virus as a vector in the environment. In particular, cross-species infection will be limited.

Most animals and birds would simply undergo an abortive infection of the orf virus, but the orf virus may still be capable of delivering an immunising dose of some antigens.

Accordingly, the narrow host range may allow the use of orf virus in animals normally resistant to infection with orf virus to stimulate an immune response. The orf virus may also be particularly useful in delivering antigens to birds, where the virus does not propagate in avian species.

WO 97/37031 PCT/NZ97/00040 - 7 -

Orf virus also has the advantage of being less virulent than vaccinia virus in man. Unlike vaccinia virus, orf virus does not cause a febrile response and lesions are shown to heal without scarring. Ideally the orf virus vector will lack its original virulence factor. Orf virus is reviewed in Robinson, A.J. and Balassu, T.C. (1981) Contagious pustular dermatitis (orf). Vet Bull 51 771-761 and Robinson, A.J. and Lyttle, D.J. (1992) "Parapoxviruses: their biology and potential as recombinant vaccines" in Recombinant Poxviruses, Chapter 9, 306-317 eds M.Binns and G. Smith CRC Press, (1992), Boca Raton.

10 The term "containing exogenous DNA" as used herein refers to exogenous DNA which is incorporated into the virus genome.

Preferably, the exogenous DNA in the orf virus vector is a gene encoding a gene product or products. The gene product may be a heterologous peptide or polypeptide but most usefully, the gene product is an antigen or antigens capable of eliciting an immune response in an infected host. Exogenous DNA encoding genes for a combination of antigens is also possible. The antigen(s) may also be treated with suitable inhibitors, modifiers, crosslinkers and/or denaturants to enhance its stability or immunogenicity if required.

20

Some examples of foreign genes of medical and veterinary importance which may potentially be incorporated into orf virus include HIV envelope protein, herpes simplex virus glycoprotein, Taenia ovis antigens, Echinococcus granulosus (hydatids) antigens, Trichostrongylus and antigens of gastrointestinal parasites such as Haemonchus and Ostertagia or combinations thereof, but are not limited thereto.

Preferred antigens include *Taenia ovis* 45W, 16kd and 18kd antigens as disclosed in WO 94/22913 incorporated herein by reference.

In a further preferred embodiment, the exogenous DNA may further comprise a cytokine gene or genes coding for other biological effector molecules which modify or augment an immune response, in combination with the exogenous antigenic DNA. Preferred cytokine genes include γ interferon and the interleukins comprising IL-1, IL-2, IL-1β, IL-4, IL-5, IL-6, IL-12 and most preferably IL-1, IL-2 and IL-12 either alone or in combination.

35

In another embodiment the exogenous DNA may further comprise one or more reporter genes and/or at least one gene coding for a selectable marker.

Examples of suitable well known reporter genes include *Escherichia coli* β -galactosidase (*lacz*), *Photinus pyralis* firefly luciferase (*lux*), secreted placental alkaline phosphatase (*SEAP*) and *Aequorea victoria* green fluorescent protein (*gfp*).

Selectable marker genes known and suitable for use in the present invention include xanthine-guanine phosphoribosyl transferase gene (xgpt), and neomycin phosphotransferase (aphII)

In a particularly preferred embodiment the exogenous DNA will comprise genes encoding multiple antigens in combination with one or more biological effector DNA molecules to enhance immune response. In practical terms where multiple antigens are coded for they will generally number 20 or less, preferably 10 or less.

Additionally, the DNA preferably encodes a peptide moiety expressed as a hybrid or chimeric protein with a native virus protein.

In this embodiment of the invention the exogenous DNA encodes for a peptide sequence that forms part of a virus protein. The native protein would retain its original properties but would exhibit additional antigenic epitopes, enzymatic properties or receptor-binding functions encoded by the exogenous DNA. Such a chimeric protein could be secreted, or could form part of the virus envelope or could form part of the virus capsid.

20

25

35

Also within the scope of the invention are fragments or variants of a vector of the invention having equivalent immunological activity. Such variants may be produced by the insertion, deletion or substitution of one or more amino acids using techniques known in the art (Sambrook, J. Fritsch, E.F. and Maniatis, T. Molecular Cloning, A Laboratory Manual (Second Edition) Cold Spring Harbour Laboratory Press 1989).

As will be appreciated by the reader, it is also desirable for the foreign gene to be incorporated into a non-essential region of the orf virus genome. In particular, the gene must be inserted into a region where it does not disrupt viral replication.

Surprisingly, the non-essential thymidine kinase gene, which is used as an insertion site in vaccinia virus has not been found in orf virus. It was therefore necessary to identify alternative non-essential sites in orf virus.

PCT/NZ97/00040

-9-

Non-essential sites were identified following restriction enzyme mapping of orf virus DNA. DNA maps for orf virus strains NZ-2, NZ-7 and NZ-10 are shown in accompanying Figure 1.

Potential insertion sites are contained within restriction fragments KpnI E of strain NZ-2, KpnI D of strain NZ-7 and KpnI D of strain NZ-10. Potential insertion sites are located in the restriction fragments BamHI E and BamHI G of strain NZ-2 shown in Figures 8 and 9. Other potential insertion sites have been identified as intergenic regions lying between regions encoding viral genes. Further examples are illustrated in Figures 4 and 5 (restriction fragments HindIII F, J, I and E of strain NZ-2) and in Figures 6 and 7 (restriction fragments BamHI F and C of strain NZ-2). Other insertion sites are also within the scope of the invention, for example, any non-essential gene or intergenic region within the orf virus genomic DNA sequence. Moreover, one or more insertion sites may be selected and used at a time.

15

WO 97/37031

There are two currently preferred insertion sites. The first of these sites is the intergenic region between RNA polymerase subunit gene, rpo132 and the open reading frame of the presumptive gene (H) I1L (Figure 4). As shown in Figure 5 this insertion site is 90 nucleotides in length, extending from positions 11 to 96.

20

The second of the preferred insertion sites is the *Nco*I site located at the beginning of gene E3L (Figure 8). As shown in Figure 9 this insertion sited is 61 nucleotides in length, extending from positions 2226 to 2286.

As will also be appreciated, if expression of the foreign gene is to be achieved, it must be under the control of a transcriptional promoter capable of expressing that gene.

A description of poxvirus promoters can be found in Moss, B. (1990). Regulation of vaccinia virus transcription. *Annu Rev Biochem.* 59, 661-688 incorporated herein by reference. As has been shown, poxvirus RNA polymerase complexes responsible for copying the gene to make a mRNA, will transcribe any gene that is preceded by a poxvirus promoter.

Preferably therefor, the promoter used will be a poxvirus promoter, and particularly a parapoxvirus promoter. The presently preferred promoter is an orf virus promoter. The orf virus promoter may be an early, intermediate or late promoter. Nucleotide sequencing has allowed the identification of a number of orf virus transcriptional promoters including

- 10 -

early, intermediate and late promoters. Orf virus early and late promoters are shown in Figure 10.

One preferred orf virus promoter is the early promoter of the putative gene E1L originally described as ORF-3 by Fraser, K. M., Hill, D. F., Mercer, A. A. and Robinson, A. J. (1990). Sequence analysis of the inverted terminal repetition in the genome of the parapoxvirus, orf virus. *Virology*. 176, 379-389 and Fleming, S. B., Fraser, K. M., Mercer, A. A. and Robinson, A. J. (1991). Vaccinia virus-like early transcriptional control sequences flank an early gene in the orf parapoxvirus. *Gene*. 97, 207-212.

10

15

Of the late promoters PF1L and PF3R are preferred. Initial studies on the relative strengths and the temporal expression of the promoters indicate that PF3R is an early-late promoter and is therefore the presently preferred promoter for expressing cloned genes encoding antigenic polypeptides. PF1L is a strong late promoter and is the presently preferred promoter for the expression of the β -galactosidase reporter gene. The orientation of the promoter and the gene it controls may be arranged as appropriate. Combinations of promoters may also be employed.

In a further aspect the invention consists in replicable transfer vectors suitable for use in preparing the modified orf virus vector of the invention. Replicable transfer vectors may be constructed according to techniques well known in the art (Sambrook, J, Fritsch, E. F. and Maniatis, T. *Molecular Cloning, A Laboratory Manual* (Second Edition) Cold Spring Harbour Laboratory Press 1989), or may be selected from cloning vectors available in the art.

25

30

The cloning vector may be selected according to the host cell to be used. Useful vectors will generally have the following characteristics:

- (i) the ability to self-replicate;
- (ii) the possession of a single target for any particular restriction endonuclease; and
- (iii) desirably, carry genes for a readily selectable marker such as antibiotic resistance.

Two major types of vector possessing the aforementioned characteristics are plasmids and bacterial viruses (bacteriophages or phages). Plasmid vectors are preferred for use in the present invention. The plasmid vector will comprise a non-essential region of the orf virus genome. a foreign gene or genes under the control of one or more orf virus

- 11 -

promoters, and a segment of bacterial plasmid DNA. The vector may be a linear DNA molecule but is preferably circular.

In the construction of a modified orf virus it is also an advantage to be able to distinguish the modified virus from the unmodified virus by a convenient and rapid assay. Such assays include measurable colour changes, antibiotic resistance and the like. For rapid assay purposes, the virus vector desirably further includes at least one reporter gene such as *lacz*, and and/or at least one selectable marker gene such as *x-gpt*.

In a preferred embodiment, the xanthine-guanine phosphoribosyltransferase gene (x-gpt) and the β-galactosidase gene are inserted into the plasmid vector under the control of suitable orf virus transcriptional promoters. The orientation of the inserted genes may also be important in determining whether recombinants can be recovered from transfections. Figure 14 shows the x-gpt gene in different orientations in pVU-DL101 and pVU-DL102.

In a further aspect, the present invention provides a method for producing a modified orf virus vector. The method comprises transfecting the plasmid cloning vectors defined above into a selected host cell infected with orf virus. Suitable transfection techniques are well known in the art, for example, calcium phosphate-mediated transfection as described by Graham, F. L. and Van der Eb, A. J. (1973). A new technique for the assay of infectivity of human adenovirus type 5 DNA. *Virology*. 52, 456-467. Other techniques include electroporation, microinjection, or liposome or spheroplast mediated transfer but are not limited thereto. Preferably, liposome-mediated transfection is used. This method is described by Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. and Danielsen, M. (1987) Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA*. 84, 7413-7417.

Upon transformation of the selected host with the cloning vector, recombinant or modified orf virus vectors may be produced. The modified virus may be detected by rapid assays as indicated above. For the preferred vectors the presence of the β-galactoside gene is detectable where clones give a blue phenotype on X-gal plates facilitating selection. Once selected, the vectors may be isolated from culture using routine procedures such as freeze-thaw extraction. Purification is effected as necessary using conventional techniques. A strategy for the generation of modified orf virus is shown in Figure 20.

The transformed host cells also form part of the invention. Many host cells are known in the art including bacterial, insect, plant and animal cells. Preferably, the host cell is a eukaryotic cell. Mammalian host cells are particularly desirable. The preferred host cells of the present invention are primary bovine testis cells or primary ovine testis cells (lamb testis cells).

5

20

25

30

As will be appreciated, in a further aspect of the invention, the protocol described above may be used to prepare heterologous polypeptides as well as antigens.

In another aspect, the present invention comprises a vaccine preparation comprising the modified orf virus which contains exogenous antigenic DNA, or a fragment or variant thereof having equivalent immunological activity thereto in combination with a pharmaceutically acceptable diluent or carrier and optionally or alternatively an adjuvant. Examples of suitable adjuvants known to those skilled in the art include saponins, Freund's adjuvants, water-in-oil emulsions, glycerol, sorbitol, dextran and many others. Generally, adjuvants will only be used with non-living viral vaccine preparations.

In a further aspect, the present invention comprises a vaccine preparation comprising the modified orf virus which contains exogenous antigenic DNA in combination with exogenous DNA encoding cytokine genes or genes for other biological effector molecules which may modify or augment an existing immune response.

The vaccine may be formulated in any convenient physiologically acceptable form. Vaccine preparation techniques for smallpox are disclosed in Kaplan, *Br. Med Bull.* 25, 131-135 (1969).

Most usefully, the vaccine is formulated for parenteral administration. The term "parenteral" as used herein refers to intravenous, intramuscular, intradermal and subcutaneous injection.

In addition the vaccine may be formulated for oral administration.

Other therapeutic agents may also be used in combination with the vaccine.

Where necessary, the vaccine may be administered several times over a defined period to maximise the antibody response to the foreign antigen.

- 13 -

Other methods for inserting foreign genes into orf virus are also contemplated. Potentially, a restriction endonuclease that cuts orf virus DNA once may be used. The cleaved site may be removed following in vitro mutagenesis followed by joining by ligation. If the site is in an essential gene the mutagenesis may be arranged such that the gene function is not affected. This is possible by substituting a base in a codon that lies wholly or partly in the restriction endonuclease cleavage site with another base that allows the new codon to code for the same amino acid but for that substitution to remove the cleavage site for that particular restriction endonuclease. The cleavage site could then be created within any non-essential gene by mutagenesis. This cleavage site then acts as a site for the insertion of foreign genes. The insertion of foreign genes may be done outside the cell by removing the phosphate from the cleaved ends of the DNA to prevent recreation of uninterrupted orf virus DNA, joining a foreign gene which has phosphorylated ends into the orf virus DNA in a ligation reaction and then transfecting the resulting ligation mixture into cells permissive for orf virus. To recover the virus the cell is infected with a poxvirus that was non-permissive for those cells, for instance fowlpox virus and primary bovine testis cells.

Non-limiting examples will now be provided.

10

15

20 Example 1 - Selection of a Suitable Cell Culture System

The source of cells for culture in the methods described in this application was calves of between one day and three months of age. The testicles were removed from the scrotum of the animal without anaesthetic by a veterinarian skilled in this procedure. The testicles were removed with the tunica parietalis intact to keep the culture cells sterile. The tissue was transported on ice to the laboratory, and the testicular tissue removed from the testis, dispersed into single cells and small aggregates of cells and incubated in suitable culture vessels in culture medium by sterile procedures familiar to those skilled in the art.

30 Example 2 - Identification of Insertion Sites

The DNAs of various orf virus isolates have been physically mapped using restriction endonucleases. Such mapping has revealed that there are many different strains of the virus that can be distinguished by the size and order of the restriction endonuclease-generated fragments although strains may not necessarily differ in their phenotype. From this data it was noted that there was a difference in size between two strains in a restriction endonuclease *KpnI* fragment mapping to the right end of the genome (Robinson A.J., Barns. G., Fraser, K. Carpenter, E. and Mercer, A.A. (1987). Conservation and

WO 97/37031 PCT/NZ97/00040 - 14 -

variation in orf virus genomes. Virology. 157, 13-23). These two strains were designated NZ-2 and NZ-7 and the fragments KpnI E and KpnI D respectively. NZ-7 contained the larger of the two fragments. The difference in size was about 1 kilobase pair. Another strain designated NZ-10 was seen to have a fragment, fragment KpnI D intermediate in size between the corresponding fragments in NZ-2 and NZ-7 but located in the same relative position in the genome (see Fig.1). This variability suggested that all or part of the region was non-essential and that within this fragment, a site in which to insert foreign DNA might be found. The regions described have subsequently been sequenced and potential insertion sites identified (Fig. 2 and Fig. 3).

10

25

Another potential insertion site was identified when DNA/DNA hybridization between strains, for example between NZ-2 and NZ-7, detected a region of non-homology extending over 2.75 kilobase pairs and this was mapped to a region about 30 kilobase pairs from the right end of the genome (Robinson A. J., Barns, G., Fraser, K, Carpenter, E. and Mercer, A. A. (1987). Conservation and variation in orf virus genomes. *Virology.* 157, 13-23 and Naase, M., Nicholson, B. H., Fraser, K. M., Mercer, A. A. and Robinson, A. J. (1991). An orf virus sequence showing homology to the fusion protein gene of vaccinia virus. *J. Gen Virol.* 72, 1177-1181) (Fig. 4). This region was then completely sequenced and two genes, HI1L and HI2L identified, each of which contains potential insertion sites (Fig. 5).

A third potential insertion site was located in the centre of the genome where a size difference of 100 base pairs was seen between the *Bam*HI G fragment in a strain designated NZ-41 and equivalent region in the other strains examined (Robinson, A. J., Barns, G., Fraser, K., Carpenter, E. and Mercer, A. A. (1987). Conservation and variation in orf virus genomes. *Virology*. 157, 13-23). The nucleotide sequence of the equivalent region in the genome of strain NZ-2, the *Bam*HI F fragment, has been determined and two potential insertion sites identified (Fig. 6 and Fig. 7).

Fourthly, a spontaneous re-arrangement of the orf virus genome of strain NZ-2 was detected following serial propagation of the virus in cell culture. This re-arrangement resulted in the addition of 16 kilobase pairs of right-end DNA sequences to the left end and the deletion of 3.3 kilobase pairs of DNA from the left end. Genomic analysis of a transposition-deletion variant of orf virus reveals a 3.3 kbp region of non-essential DNA (Fleming, S. B., Lyttle, D. J., Sullivan, J. T., Mercer, A. A. and Robinson, A. J. (1995). J Gen Virol., 76, 2969-2978). The order of nucleotides making up the region of the genome that can tolerate a deletion has been deduced by the method of Sanger and three genes contained therein identified. These genes correspond to E2L, formerly ORF-1

- 15 -

(Fraser, K. M., Hill, D. F., Mercer, A. A. and Robinson, A. J. (1990). Sequence analysis of the inverted terminal repetition in the genome of the parapoxvirus, orf virus. *Virology.* 176, 379-389), E3L formerly ORF-PP (Mercer, A. A., Fraser, K., Stockwell, P. A. and Robinson, A. J. (1989). A homologue of retroviral pseudoproteases in the parapoxvirus, orf virus. *Virology* 172, 665-668) and G1L (Sullivan, J. T., Fraser, K., Fleming, S. B., Robinson, A. J. and Mercer, A. A. (1995). Sequence and transcriptional analysis of an orf virus gene encoding ankyrin-like repeat sequences. *Virus Genes*, 9, 277-282). This region (Fig. 8) is another potential site for gene insertion (see Fig. 9).

10 Example 3 - Identification of Orf Virus Promoters

15

20

25

30

Determining the nucleotide sequence of selected regions of the orf virus genome has allowed the identification of a number of orf virus transcriptional promoters, in the first instance by virtue of their similarity to other poxvirus transcriptional promoters, and later by functional assays.

Orf virus early and late promoters are shown in Figure 10. The early promoter E1L (ORF-3) was shown to make mRNA early in the cell cycle (Fleming, S. B., Fraser, K. M., Mercer, A. A. and Robinson, A. J. (1991). Vaccinia virus-like early transcriptional control sequences flank an early gene in the orf parapoxvirus. *Gene.* 97, 207-212) and the late promoter F1L was deduced to be a late promoter by virtue of its similarity to a vaccinia virus late promoter. The orf virus late promoter is functional in a transient assay. Such assays have been described for instance by (Cochran, M. A., Mackett, M. and Moss. B. (1985). Eukaryotic transient expression system dependent on transcription factors and regulatory DNA sequences of vaccinia virus. *Proc Natl Acad Sci USA*. 82, 19-23). A third promoter F3R, identified as an early-late promoter, is also shown to be functional in a transient assay. The construction of a plasmid pSP-PFlac containing the orf virus late promoter, F1L, and the *E. coli* gene for \(\beta\)-galactosidase (lacz) such that the \(\beta\)-galactosidase gene is under the control of the orf virus late promoter is described in Example 6 and illustrated in Figure 11.

(A) Assessment of Promoter Activity in Transient Assay

To show that the promoter is active in a transient assay, a confluent monolayer of bovine testis cells, in a plastic flask of 25 cm2 surface area for the adherence of the cells and suitable for cell culture work, was infected with orf virus at a multiplicity of infection of approximately 10 plaque forming units per cell. Two hours after infection, the plasmid containing the *lacz* gene linked to the promoter under investigation was introduced into

- 16 -

orf virus infected bovine testis cells using the liposome mediated transfer technique as described by (Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. and Danielsen, M. (1987). Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA*. 84, 7413-7417) and as set forth in Example B. Forty eight hours after infection, 35 µl of a solution of 5-bromo-4-chloro-3-indolyl-\beta-D galactosidase (X-gal) at a concentration of 2% w/v in water was added to 1 ml of 1% agarose in cell culture medium which was overlayed onto the cells after the removal of the liquid medium and allowed to form a gel at room temperature (in the range of 15°-25° C). Over the succeeding 24 hours the development of a blue coloration in the cells and in the gel above the affected cells was looked for. The development of a blue coloration greater than that seen in cells treated similarly, but with a plasmid containing the \beta-galactosidase gene not under control of a transcriptional promoter, indicated that the promoter being tested was active.

In a further aspect of investigating promoter function a quantitative assay for β galactosidase activity in transiently-infected bovine testis cells is performed. Cells are grown as confluent monolayers in multiwell plastic tissue culture trays containing 24 wells 1.5 cm in diameter. Individual wells are infected with orf virus at a moi of 10 and two hours after infection the plasmid construct containing the promoter linked to the βgalactosidase gene is introduced into the infected cells using the liposome mediated transfection technique described above. Cells are harvested by scraping into a 1 ml volume of phosphate-buffered saline (PBS), collected by centrifugation, washed with PBS and resuspended in a 200µl volume of PBS. Cells are disrupted by three cycles of freezing and thawing, centrifuged, and the supernatant retained for the enzyme assay. The assay for \u03b3-galactosidase is conveniently performed in 96-well microtitre trays. The reaction mixture of 0.1 ml contains 100mM Na-phosphate, pH 7.3, 1mM MgCl2, 50mM β -mercaptoethanol, O-nitrophenyl- β -D-galactoside (ONPG) at a final concentration of 1.3mg/ml and a 10-20µl aliquot of the cell lysate. The reaction mix is incubated at 370 C for 1 hour and the reaction is terminated by the addition equal volume of 1M NaCO3. The absorbance of each well is measured at 420 nm using a microtitre plate reader. The absorbance value is proportional to the amount of B-galactosidase activity present in the original extract and this enables the time course of expression and the relative strength of each promoter construct to be determined.

30

10

15

20

Example 4 - Construction of a Vector Plasmid Suitable for the Insertion of Foreign Genes into the Orf Virus Genome

The choice of non-essential DNA was the region discovered to be deleted in a re-arranged mutant of orf virus and the relevant sequence of nucleotides in this region can be found in Fraser, K. M., Hill, D. F., Mercer, A. A. and Robinson, A. J. (1990). analysis of the inverted terminal repetition in the genome of the parapoxvirus, orf virus. Virology. 176, 379-389 and in Sullivan, J. T., Fraser, K. M., Fleming, S. B., Robinson, A. J. and Mercer, A. A. (1995). Sequence and transcriptional analysis of an orf virus gene encoding ankyrin-like repeat sequences. Virus Genes 9, 277-282 and is shown in Figure 8. The orf virus promoters used were an early promoter, E1L, described in Fraser, K. M., Hill, D. F., Mercer, A. A. and Robinson, A. J. (1990). Sequence analysis of the inverted terminal repetition in the genome of the parapoxvirus, orf virus. Virology. 176, 379-389 and Fleming, S. B., Fraser, K. M., Mercer, A. A. and Robinson, A. J. (1991). Vaccinia virus-like early transcriptional control sequences flank an early gene in the orf virus. Gene. 97, 207-212 and a late promoter F1L (Fleming, S. B., Blok, J., Fraser, K. M., Mercer, A. A. and Robinson, A. A. (1993). Conservation of gene structure and arrangement between vaccinia virus and orf virus. Virology. 195, 175-184) as shown in Figure 10. The foreign genes chosen to demonstrate the process of creating a mutated orf virus were the E. coli B-galactosidase gene, which has the advantage that when expressed the protein product can be detected by a colour reaction (Miller, J. H. (1972). "Experiments in Molecular Genetics." Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Moss, B. (1990). "Poxviridae and their Replication" in Virology. Fields et al., eds, 2nd ed. Raven Press, New York, 2079-2111), and the E. coli guanyl phoshoribosyl transferase (x-gpt) gene which when expressed can be used to select mutants from unmutated virus (Mulligan, R. C. and Berg, P. (1980). Expression of a bacterial gene in mammalian cells. Science. 209, 1422-1427). The following is a description of the construction of the vector plasmid. Figures 11 -13 outline the construction in diagrammatic form.

20

25

30

35

(A) Cloning an Orf Virus Late Promoter in Front of the E. coli LacZ Gene

In the construction of a mutant orf virus it is an advantage to be able to distinguish mutant virus from unmutated virus by a convenient and rapid assay. Such an assay is provided by inserting the *E. coli* gene for the \(\beta\)-galactosidase enzyme under control of an orf virus transcriptional promoter into the vector plasmid. The late orf virus promoter was identified by determining the nucleotide sequence of a fragment of orf virus DNA designated \(\beta am \)HIF (Fleming. S. B., Blok, J., Fraser, K. M., Mercer, A. A. and Robinson.

WO 97/37031 PCT/NZ97/00040 - 18 -

A. A. (1993). Conservation of gene structure and arrangement between vaccinia virus and orf virus. Virology. 195, 175-184). The sequence of the promoter F1L used in this construction is shown in Fig. 10. A sufficient quantity of the late promoter for the construction can be obtained from the plasmid designated pVU-6 which has been described (Mercer, A. A., Fraser, K., Barns, G. and Robinson, A. J. (1987). The structure and cloning of orf virus DNA. Virology. 157, 1-12). A total of 2.62 kb of DNA is deleted from the BamHI F fragment of orf NZ-2 by digesting the plasmid pVU-6, which contains the BamHI F fragment of orf NZ-2 cloned into the plasmid pUC-8 (Viera, J. and Messing, J. (1982). The pUC plasmids, an M13mp7 derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene. 19, 259-268) with Aval. This enzyme cleaves the Smal site of the pUC-8 polylinker and six internal Aval sites in BamHI E. The Aval sites remaining on the vector fragment are end-filled with Klenow DNA polymerase, and religated to give the plasmid pVU-Av6. The plasmid pVU-Av6 is cut with BamHI and EcoRI releasing a 725 bp fragment containing the orf virus late promoter. This fragment is cloned into pMLB 1034 (Weinstock, G. M., Berman, M. L. and Silhavy, T. J. (1983). "Chimeric genetics with \(\beta\)-galactosidase in gene amplification and analysis." in Expression of Cloned Genes in Procaryotic and Eucaryotic Cells, Papas et al., eds. Elsevier, New York, 27-64) which contains a "headless" lacz gene. This cloning places the orf virus late promoter in front of lacz and supplies it with an ATG initiation codon allowing the synthesis of B-galactosidase. The colonies that result from this cloning step give a blue phenotype on X-gal plates facilitating the selection of the required clone. A unique Ball site downstream from the lacz insert of pMLB-1034 is converted to an EcoRI site by the following cloning steps. The Tn5 aminoglycoside 3' phosphotransferase gene is released from the plasmid pNEO (Beck, E., Ludwig, A., Aurswald, E. A., Reiss, B. and Schaller, H. (1982). Nucleotide sequence and exact location of the neomycin phosphotransferase from transposon Tn5. Gene. 19, 327-336) with EcoRI and BamHI. The restriction sites are end-filled with Klenow DNA polymerase and the fragment ligated into plasmid pMLB-PF which had been cut with Ball. Recombinants are selected by plating on kanamycin medium. This creates an EcoRI or BamHI site at the position of the original BalI site depending on the orientation of the cloned aminoglycoside 3'-phosphotransferase II (aphII) gene. Ball often cuts DNA inefficiently, but the method allows for the selection of the plasmids which have been cut by Ball and have received the insert, consequently becoming modified in the desired manner. The plasmid pMLB-PFneo is cut with EcoRI and a 4059 bp EcoRI fragment containing the PF-lacZ fusion is cloned into pSP-70 (Melton, D. A., P.A., R., Rebagliati, M. R., Maniatis, T., Zinn, R. and Green, M. R. (1984). Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing

10

15

20

25

- 19 -

bacteriophage SP6 promoter. Nucleic Acids Res. 12, 7035-7056) at the EcoRI site to give the plasmid designated pSP-PFlac shown in the diagram Fig. 11.

(B) Cloning of an Orf Virus Early Promoter in Front of the E. coli X-GPT Gene

5

10

15

20

25

In the construction of the mutated orf virus, a means of selecting mutants from nonmutants, from a mixture of both, is required. A method that has been used by others is to utilise the guanyl phosphoribosyl transferase gene of E. coli. Resistance is conferred to a metabolic inhibitor, mycophenolic acid, when the gene is expressed in a eukaryotic cell. A method for incorporating this gene into a vector plasmid under the control of an early promoter is described by Falkner, F. G. and Moss, B. (1988). Escherichia coli gpt gene provides dominant selection for vaccinia virus open reading frame expression vectors. J Virol. 62, 1849-1854 and Boyle, D. B. and Coupar, B. E. (1988). Construction of recombinant fowlpox viruses as vectors for poultry vaccines. Virus Res. 10, 343-356. A plasmid designated pVU-5 is used to provide an early orf virus promoter. The plasmid pVU-5 contains the orf virus NZ-2 BamHI E fragment cloned into pUC-8 and the construction of this plasmid is described in Mercer, A. A., Fraser, K., Barns, G. and Robinson, A. J. (1987). The structure and cloning of orf virus DNA. Virology. 157, 1-12. An early promoter E1L has been described for the putative gene originally designated ORF-3 in pVU-5 by Fraser, K. M., Hill, D. F., Mercer, A. A. and Robinson, A. J. (1990). Sequence analysis of the inverted terminal repetition in the genome of the parapoxvirus, orf virus. Virology. 176, 379-389 and by Fleming, S. B., Fraser, K. M., Mercer, A. A. and Robinson, A. J. (1991). Vaccinia virus-like early transcriptional control sequences flank an early gene in the orf parapoxvirus. Gene. 97, 207-212; and it is this early promoter that is used in the method described in this application to construct a mutant orf virus. A 503 bp Alul A+T-rich fragment shown in the Fig. 12 is cleaved from pVU-5 and cloned into the HincII site of the multifunctional plasmid vector pTZ18R described in Mead, D. A., Szczesna-Skorupa, E. and Kemper, B. (1986). Single-stranded DNA "blue" T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. Protein Eng. 1, 67-74 giving pSFAlu-6. Plasmid pSFAlu-6 is cut with DdeI and the fragments 30 end-filled with Klenow DNA polymerase. The fragments are recut with HindIII and a 467 bp HindIII- DdeI fragment ligated into pSP-70 which is prepared by cutting with Bg/II, end-filling and recutting with *Hind*III. The resulting plasmid pSP-SFP retains the *Bgl*II site which is reformed during the cloning step. The plasmid pSV-gpt2, containing the E. 35 coli x-gpt gene, (Mulligan, R. C. and Berg, P. (1981). Selection for animal cells that express the Escherichia coli gene coding for xanthine-guanine phosphoribosyl transferase. Proc Natl Acad Sci USA. 78, 2072-2076) is cut with BamHI and BglII. This releases the x-gpt gene as a 1788 bp fragment which is then cloned into the BglII site of pSP-SFP.

fusing the orf virus fragment to the x-gpt gene giving pSP- SFPgpt32. The plasmid pVU-5 is then cut with Smal and Sphl. A 150 bp Smal-Sphl fragment containing the early promoter E1L, the sequence of which is shown in Fig. 10, is cloned into pTZ18R between the Smal and SphI sites giving the plasmid pFS-1. The plasmid pFS-1 is cut with SphI and incubated with T4 DNA polymerase. The aphII gene is released from the plasmid pNEO with EcoRI and BamHI. The EcoRI and BamHI sites are end-filled with Klenow DNA polymerase and the fragment ligated into pFS-1. The resulting plasmid pFS-neo3 contains the aphII gene flanked by an EcoRI site and a BamHI site which lies between it and the early orf virus promoter. A result of these manipulations is that the SphI site distal to the early promoter is converted to a BamHI site. The aphII gene and the early promoter lie in a "head-to-head" orientation and may be removed by digestion with EcoRI. Next, the plasmid pSP-sSFPgpt32 is cut with PvuII. The aphII-early promoter construct was cut out of pFSneo3 with EcoRI, end-filled with Klenow DNA polymerase, and ligated into the PvuII site. A plasmid termed FSneo-SFPgpt which contains the early promoter running in the same direction as the 503 bp Alul fragment is selected. The plasmid FSneo-SFPgpt is cut with BamHI and BglII. This step removes the sequence between nucleotides a and b (Fig. 13) together with the aphII gene as a BamHI-BglII fragment. The vector fragment is subjected to electrophoresis in an agarose gel and then purified using the powdered glass milk method described by (Vogelstein, B. and Gillespie, D. (1979). Preparation and analytical purification of DNA from agarose. Proc Natl Acad Sci USA. 76, 615-619) and the free BamI and BglII termini ligated together fusing the early promoter to the x-gpt gene. The net result of the manipulations described in steps 4, 5, 6, and 7 (Fig. 13) was to replace the sequence between nucleotides a and b in pSP-SFPgpt32 with the FS promoter forming pFS-gpt.

10

15

20

25

30

35

Example 5 - Identification of a Non-essential Region of the Orf Virus Genome and Insertion of this Site into a Plasmid

A gene coding, potentially, for a peptide of 159 amino acids was found from the sequencing of the 4.47 kb BamHI E fragment which spans the ITR junction of the orf virus genome. This was termed E3L (ORF-PP) and shows homology to an open reading frame in retroviruses (Mercer, A. A., Fraser, K. M., Stockwell, P. A. and Robinson, A. J. (1989). A homologue of retroviral pseudoproteases in the parapoxvirus, orf virus. Virology. 172, 665-668) and to E. coli dUTPase (McGeoch, D. J. (1990). Protein sequence comparisons show that the 'pseudoproteases' encoded by poxviruses and certain retroviruses belong to the deoxyuridine triphosphatase family. Nucleic Acids Res. 18, 4105-4110). A spontaneous mutant of orf virus isolated in the laboratory was found not to contain the E3L gene due to a complex rearrangement involving the deletion of part of

- 21 -

the BamHI E fragment and duplication of DNA segments from the opposite end of the genome at that locus. The E3L gene is therefore non-essential and was chosen as a target for the insertion of foreign DNA and to demonstrate that orf virus could tolerate the insertion of a foreign gene. A 2587 bp SmaI-BamHI fragment (Fig. 14) containing the unique region of NZ-2 BamHI E is cut out of pVU-5 and cloned into pSP-70 cut with PvuII and Bg/II. The resulting plasmid, pVU-DL100 contains a unique NcoI site that lies between the coding sequence of the E3L gene and its promoter.

Example 6 - Insertion of the E. coli X-GPT and Lac Z Gene Constructs into pVU-DL100 to Create a Vector Plasmid

10

15

20

Plasmid pVU-DL100 is cut with Ncol and end-filled with Klenow polymerase. The E3L-gpt construct is cut from pFSP-gpt with EcoRI and DraI, end-filled with Klenow polymerase and ligated into pVU-DL100 at the Ncol site. Ligation of the end-filled EcoRI site of the insert to the end-filled Ncol site on the plasmid creates an EcoRI site upstream of the early promoter. The insert is recovered in two orientations, pVU-DL101 with the x-gpt gene running in the opposite direction to the pseudoprotease gene and pVU-DL102 with the x-gpt gene running in the same direction as the pseudoprotease gene. The F1L-lac construct is cut out of pSP-PFlac with EcoRI and cloned into the EcoRI sites of both pVU-DL101 and pVU-DL102. Four plasmids with different orientations of the inserted fragments are recovered from the cloning but only two, pVU-DL104 derived from pVU-DL101, and pVU-DL106 derived from pVU-DL102 which contain the E3L-gpt and F1L-lac in the "back-to-back" orientation are used for transfection experiments.

25 Example 7- Constructing a Chimeric Gene Expressing the T. ovis 45W antigen.

A 64 bp fragment of the VEGF like-gene from orf virus NZ-7 (Lyttle, D. J., Fraser, K. M., Fleming, S. B., Mercer, A. A. and Robinson, A. J. (1993) Homologs of vascular endothelial growth factor are encoded by the poxvirus orf virus. J Virol. 68, 84-92) containing five 3' prime terminal codons, the translational termination codon TAA, and a poxvirus transcriptional terminator sequence 5TNT, was amplified using a pair of oligonucleotide primers designed to provide a Bg/II and a Ncol restriction site flanking the amplified sequence. This fragment was digested with Bg/II and Ncol and ligated into the vector pSL301 (Brosius, J. (1989) Superlinkers in cloning and expression vectors. DNA 8, 759-777) cut with Bg/III and Ncol to form the plasmid ptov1. A DNA fragment containing the aph/II gene and the F1L and F3R promoters of orf virus was amplified by PCR using specific primers which introduced a MluI site at one end and a NsiI and EcoRI site at the other end. One portion of the amplified product was digested with MluI and

EcoRI and ligated into ptov1 cut with MluI and EcoRI to create the plasmid ptov2. A second portion was digested with MluI and NsiI and ligated into ptov1 to form the plasmid ptov3. The steps showing this construction are illustrated in Figure 15.

The aphII gene was removed from the plasmid ptov2 by digesting with the restriction enzymes BamHI and BglII, purifying the vector fragment and re-ligating the free ends to form the plasmid ptov5. The DNA sequence encoding the Taenia ovis 45W antigen fragment was removed from the plasmid pGEX 45W (Johnson, K. S., Harrison, G. B. L., Lightowlers, M. W., O'Hoy, K. L., Cougle, W. G., Dempster, R. P., Lawrence, S. B., Vinton, J. G., Heath, D. D., and Rickard, M. D. (1989). Vaccination against ovine cysticercosis using a defined recombinant antigen. (Nature 338, 585-587) by digesting with the restriction enzymes EcoRI and Bam HI and ligating it into ptov5 cut with BamHI and EcoRI to form ptov6. This placed the DNA sequence encoding the 45W antigen fragment under the control of the orf virus PF3R promoter and supplied it with translational and transcriptional termination sequences. These steps are illustrated in Figure 16.

A 73 bp fragment from the 5' portion of the VEGF-like gene from orf virus NZ-7 encoding the presumptive secretory leader sequence was amplified with specific primers which introduced a new initiation codon, a PstI and an EcoRI restriction site into the amplified DNA fragment. The amplified fragment was digested with PstI and EcoRI and cloned into ptov3 cut with NstI and EcoRI to create the plasmid ptov4. The plasmid ptov4 was digested with BamHI to remove the aphlI gene, purified by agarose gel electrophoresis and religated to form the ptov7. The DNA sequence encoding the 45W antigen fragment was removed from the plasmid pGEX 45W by digesting with the restriction enzymes EcoRI and Bam HI and ligating it into ptov7 cut with BamHI and EcoRI to form ptov8. This placed the 45W antigen fragment under the control of the orf virus PF3R promoter and supplied a 5' protein secretory leader sequence in addition to the 3' translational and transcriptional terminators present in ptov6. These steps are illustrated in Figure 17.

20

25

30

35

The plasmid pVU-DL101 was cut with EcoRI and an oligonucleotide linker containing a BamHI and a NcoI restriction site was ligated in to form the plasmid pVU DL101L4. This plasmid was then digested with BamHI and NcoI to allow the insertion of both versions of the chimeric 45W gene from ptov6 and from ptov8. The resulting plasmids were designated pVU-dl45W (from ptov6) and pVU-dl45Wl (from ptov8). These steps are illustrated in Figure 18.

A promoterless *lacz* gene was cleaved out of the plasmid pVUsp-PF2lac, a derivative of pSP PFlac illustrated in Fig.11 by digestion with BamHI and BgIII. In this latter version of the plasmid, the F1L promoter fragment has been truncated to 100 base pairs and a *BgI*II restriction site introduced distal to the *lacz gene*. The *lacz* fragment was gel purified and ligated into both pVU-DL45W and pVU-Dl45Wl at a unique *Bam*HI site. This placed the *lacz* gene under the control of the F1L promoter and completed the construction of the transfer vectors for introducing the *T. ovis* 45W gene into the orf virus genome. These steps are illustrated in Figure 19.

10 The same oligonucleotide linker containing the BamHI and a NcoI restriction sites was ligated into the plasmid pVU-DL102. This plasmid contains the x-gpt gene cloned in the opposite orientation to that in pVU-DL101 (Fig 14). Cloning steps parallel to those described for pVU-DL101 were subsequently performed and the transfer vectors which were generated were designated pVU-DL45W6lac and pVU-DL45W8lac. These contained the same sequences as pVU-DL45Wlac and pVU-DL45Wllac respectively, but differed in that the entire inserted region was in the opposite orientation to that illustrated for these plasmids in Fig. 19.

Example 8 - Transfection Protocol

20

25

Primary bovine testis (BT) cells were grown in monolayer cultures in Eagle's Minimal Essential Medium (MEM; Sigma Cat. No. M0643) supplemented with lactalbumin hydrolysate (5 g/L) and 5% foetal calf serum. Medium for selecting orf virus transformants expressing *x-gpt* contain mycophenolic acid, 25 μg/ml, xanthine, 250 μg/ml, hypoxanthine, 15 μg/ml, aminopterin, 1 μg/ml, thymidine, 5 μg/ml and 2% foetal calf serum. Lactalbumin hydrolysate was omitted from the selective medium and replaced with additional non-essential amino acids (MEM non-essential amino acid mixture, Sigma Cat. No. M2025).

BT cells were grown as monolayers in a suitable cell culture vessel. Twenty-four hours prior to infection, the cell growth medium was replaced with the selective medium containing mycophenolic acid. The cells were infected with orf virus, strain NZ-2, (moi 0.05 - 0.1) and the virus allowed to adsorb for 1 hour. Cell monolayers were washed 2 times with opti-MEM serum-free medium, (Life Technologies Inc, Gaithersburg, MD U.S.A.) to remove residual foetal calf serum, and drained. A 1.0 ml volume of opti-MEM containing 10. µl Lipofectin reagent (Life Technologies Inc, Gaithersburg, MD, U.S.A.) and approximately 2.0 µg plasmid DNA diluted according to the suppliers instructions was added to each flask and incubated overnight. Following this overnight adsorption

step, 5.0 ml of selective medium containing 2% foetal calf serum was added and the incubation continued until cytopathic effect (CPE) was observed approximately 3 - 5 days post-infection.

- 24 -

Cell monolayers were scraped from the flask, deposited in the bottom of a centrifuge tube by low speed centrifugation, washed with phosphate buffered saline (PBS) and resuspended in PBS. A suitable tissue culture vessel was seeded with BT cells to produce a confluent monolayer. Routinely, 60mm diameter polystyrene dishes were used, seeded with 1.5 x 106 cells per dish and incubated in a CO₂ atmosphere to maintain a pH of around 7.2. The culture medium was removed and 0.5 ml of an appropriate dilution of 10 orf virus in PBS was added and incubated for one hour at 37°C. Dishes were tipped at 15 min intervals to ensure an even distribution of fluid. At the end of this time the inoculum was removed and growth medium containing 1% agarose added. After five days, the time when plaques usually become visible, X-gal was added to the dish in a 1% agarose overlay and incubated a further 12 hours for colour development to occur. Single plaques 15 are picked, resuspended in PBS and inoculated into a partially drained cell culture vessel which had been seeded with 2 x 105 cells and grown to confluence as described. One ml of medium was added to each well and incubation at 37°C continued until a complete cytopathic effect was observed. The cell culture vessels were placed at -20°C until the contents were frozen after which time they were thawed. The cell lysates were used as 20 a source of virus, for further plaque purification, and of viral DNA for hybridisation. Viral DNA was prepared from cytoplasmic extracts of BT cells by the method of Moyer, R. W. and Graves, R. L. (1981). The mechanism of cytoplasmic orthopoxvirus DNA replication. Cell. 27, 391-401. The isolated DNA was digested with restriction enzymes to confirm the insertion of the foreign genes. Frequently, the first plaque purification step 25 fails to remove all the wild type virus and a series of plaque purification steps may be performed in order to obtain a pure culture of mutated virus. Bulk cultures of virus are grown in 150 cm2 tissue culture flasks and the virus purified by the method described in Robinson, A. J., Ellis, G. and Balassu, T. (1982). The genome of orf virus: restriction endonuclease analysis of viral DNA isolated from lesions of orf in sheep. Arch Virol. 71, 43-55. DNA is extracted from the purified virions by the method described in Balassu, T. C. and Robinson, A. J. (1987). Orf virus replication in bovine testis cells: kinetics of viral DNA, polypeptide, and infectious virus production and analysis of virion polypeptides. Arch Virol. 97, 267-281.

- 25 -

Example 9 - Assessment of Orf Virus Modification

20

25

30

35

In order to determine whether or not the viruses recovered from the transfections and plaque purifications were modified to carry the inserted genes, DNA was prepared from infected cells and tested by hybridisation by methods well known to those skilled in the art, for example, Merchlinsky, M. and Moss, B. (1989). Resolution of vaccinia virus DNA concatemer junctions requires late-gene expression. J Virol. 63, 1595-1603. In the preparation of mutated orf virus DNA for these tests, a 100 µl aliquot of orf virus-infected BT cells in PBS was centrifuged for 30 min at approximately 12,000g. The cell pellet was resuspended in 50 µl 0.15M NaCl, 20mM Tris, 10 mM EDTA, pH 8.0. A 250 µl volume of 20mM Tris, 10mM EDTA, 0.75% SDS containing a protease at an appropriate concentration (e.g. Proteinase K at 0.5 mg/ml) was added to each sample and incubated at 370 C for 3 hours. The samples were extracted with an equal volume of phenol:chloroform (1:1) before precipitation with ethanol. Following centrifugation the ethanol-precipitated DNA was redissolved in 50 µl TE. The material harvested from the various passages was subjected to the hybridization procedure with a specific x-gpt probe. A positive result can be obtained with pVU-DL106 for the transfection two hours postinfection as early as passage one. An alternative procedure that was used to detect heterologous DNA markers in recombinant virus was to amplify DNA sequences by the polymerase chain reaction using primers specifically designed to amplify the foreign DNA sequences. Other transfections may require further passages for the detection of recombinant viruses. Transfections performed with the plasmid pVU-DL106 at two hours allowed CPE to be detected at three days post-inoculation at passage three and the detection of mutated virus containing the x-gpt gene as determined by DNA-DNA hybridization. A qualitative assay for B-galactosidase activity using the chromogenic substrate 5-bromo-4-chloro-3-indolyl-B-D-galactoside (X-gal) was used to detect mutated orf virus containing the \(\beta\)-galactosidase gene.

Example 10 - Construction of a vector plasmid suitable for the insertion of foreign genes into the region of the orf virus genome corresponding to the orthopoxvirus ATI-region

The intergenic region between the RNA polymerase subunit gene, rpo 132 and the open reading frame of the presumptive gene (H)I1L was identified as a suitable target site for the insertion of foreign DNA. The region is 90 nucleotides in length and lies between two converging transcriptional elements one of which, rpo 132, is an essential gene. A plasmid, PB-23ΔSal, which contains a sequence of 1.6 kilobases extending into the unsequenced region upstream of position 1 shown in the sequence illustrated in Figure 5 and terminating at the *Pst*I site at position 178 was used as the template in a PCR cloning

WO 97/37031 - 26 -

PCT/NZ97/00040

reaction. A sequence of 1.0 kb was amplified from it using the primers zxs-1 GATCCCGCTCGAGAACTTCAA (forward) which is complementary to a sequence identified in PB-23ΔSal that contains an existing XhoI restriction site and zxs-2 GTCAGATCTATGCATAAAAATTTCGCATCAGTCGAGATA (reverse) which introduces a BglII, a NsiI and an ApoI restriction site. The amplified fragment was purified by electrophoresis on a 1% agarose gel and digested with the restriction enzymes XhoI and BglII. The purified fragment was ligated then into the plasmid pSP-70 at the corresponding XhoI and BglII sites creating the plasmid pTvec1. This cloning step also introduced a poxvirus transcriptional termination signal (5TNT) into the vector.

10

15

20

35

5

A second fragment comprising the sequence located between nucleotide positions 66 and 1069 (Fig 5 was amplified with the primers zxs-3 GACATGCATCAGTGCCATGGAATTCTCGCGACTTTCTAGC (forward) which sites **Eco**RI restriction zxs-4 introduces NsiI, NcoI and and GACGGATCCGTATAATGGAAAGATTC (reverse) which introduces a BamHI restriction site. The amplified fragment was digested with the restriction endonucleases BamHI and NsiI and purified in the same manner as the first fragment. The purified fragment was then cloned into pTvec1 which had been cut with NsiI and BglII. The resulting plasmid pTvec50 contains a series of restriction sites and a transcriptional termination signal which are available for further cloning steps. These restriction sites are Apol, Nsil, Ncol and EcoRl. The sequence of the primers, the restriction sites and the sequence of the modified intergenic region are shown in Figures 20A and 20B. The cloning steps involve in the construction of ptvec50 are illustrated in Figure 21.

A lacz gene under the control the orf virus late promoter PF1L was cleaved out of the plasmid pVUsp-PF2lac with EcoRI. The fragment was gel purified and ligated into the EcoRI site of pTvec50. Recombinant plasmids containing the lacz gene in both possible orientations were recovered and designated pTvec50lac-1 and pTvec50lac-2. The cloning steps involved in the construction of pTvec50lac-1 and pTvec50lac-2 are illustrated in Figure 22. This completed the construction of a transfer vector designed to introduce the foreign gene lacz into the intergenic site between the open reading frames of rpo 132 and (H)11L shown in Fig 5.

In this example the *xgpt* gene was not included in the transfer vector and consequently selection of recombinant orf virus expressing *xgpt* by growth in the presence of mycophenolic acid was not able to be used as a selection method. Virus recombinants were selected by using *lacz* expression as the primary method for identifying

recombinants containing an insertion in the ATI region. The following variation of the method described in Example 8 was used.

Primary bovine testis (BT) cells were grown in monolayer cultures in Eagle's Minimal Essential Medium (MEM); (Sigma Cat. No. M0643) supplemented with lactalbumin hydrolysate (5 g/L) and 5% foetal calf serum. Prior to infection the cell growth medium was removed and the cells washed briefly with phosphate buffered saline (PBS) to remove residual serum. The cells were infected with orf virus, strain NZ-2, (moi 0.05 - 0.1) and the virus allowed to adsorb for 1 hour. Cell monolayers were washed 2 times with opti-MEM serum-free medium, (Life Technologies Inc, Gaithersburg, MD, U.S.A.) to remove non-adsorbed virus and residual foetal calf serum, and drained. A 1.0 ml volume of opti-MEM containing 10 µl Lipofectin reagent (Life Technologies Inc, Gaithersburg, MD, U.S.A.) and approximately 2.0 µg plasmid DNA diluted according to the suppliers instructions was added to each flask and incubated overnight. Following this overnight adsorption step, 5.0 ml of selective medium containing 2% foetal calf serum was added and the incubation continued until cytopathic effect (CPE) was observed approximately 3 -5 days post-infection.

Cell monolayers were scraped from the flask, deposited in the bottom of a centrifuge tube by low speed centrifugation, washed with PBS and resuspended in PBS. The resuspended cells were subjected to three cycles of freezing and thawing and sonicated briefly. The virus titre of the harvested culture was determined and the material plated on fresh dishes of BT cells at a dilution calculated to give approximately 2000 virus plaques per dish. Sufficient material was plated to screen 50,000 plaques (25 dishes). The infected monolayers were grown under an a 1% agarose overlay and after 5 days incubation when plaques became visible, X-gal in a 1% agarose overlay was added to the dishes and incubated a further 12 hours for colour development to occur. At this stage, any coloured plaques which had appeared were picked and treated as described in Example 8. Further purification of the recombinant virus was achieved by repeated cycles of plating and picking single, coloured plaques until a pure culture of *lacz* positive virus was obtained.

APPLICATION OF THE INVENTION

10

15

20

25

30

In accordance with the present invention there is provided a parapoxvirus vector, specifically an orf virus vector, containing exogenous DNA. The exogenous DNA may encode an antigen capable of inducing an immune response or may encode a heterologous polypeptide of which expression is desired.

The vectors of the present invention therefore have particular applications in the expression of heterologous polypeptides and antigens. The capacity to express antigens make these vectors particularly suitable for use in vaccines.

- 28 -

- Orf virus vectors have a number of advantages over vaccinia virus vectors. Orf virus has a relatively narrow host range compared to vaccinia. This reduces the vaccinia associated risks of cross-species infection and spread of disease. A further advantage is that orf virus is less virulent than vaccinia in man, reducing the risks of febrile response and lesions.
- 10 It will be appreciated that the above description is provided by way of example only and that the invention is limited only by the scope of the appended claims.

References

Balassu, T. C. and Robinson, A. J. (1987). Orf virus replication in bovine testis cells: kinetics of viral DNA, polypeptide, and infectious virus production and analysis of virion polypeptides. *Arch Virol.* 97, 267-81.

Beck, E., Ludwig, A., Aurswald, E. A., Reiss, B. and Schaller, H. (1982). Nucleotide sequence and exact location of the neomycin phosphotransferase from transposon Tn5. *Gene.* 19, 327-336.

10

Boyle, D. B. and Coupar, B. E. (1988). Construction of recombinant fowlpox viruses as vectors for poultry vaccines. *Virus Res.* 10, 343-56.

Brosius, J. (1989) Superlinkers in cloning and expression vectors. DNA 8 759-777

15

Cochran, M. A., Mackett, M. and Moss, B. (1985). Eukaryotic transient expression system dependent on transcription factors and regulatory DNA sequences of vaccinia virus. *Proc Natl Acad Sci U S A.* 82, 19-23.

Falkner, F. G. and Moss, B. (1988). Escherichia coli gpt gene provides dominant selection for vaccinia virus open reading frame expression vectors. *J Virol.* 62, 1849-54.

Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. and Danielsen, M. (1987). Lipofection: a highly efficient, lipid-mediated DNA- transfection procedure. *Proc Natl Acad Sci USA*. 84, 7413-7417.

Fleming, S. B., Fraser, K. M., Mercer, A. and Robinson, A. J. (1991). Vaccinia virus-like early transcriptional control sequences flank an early gene in the orf parapoxvirus. *Gene.* 97, 207-212.

30

Fleming, S. B., Blok, J., Fraser, K. M., Mercer, A. A. and Robinson, A. A. (1993). Conservation of gene structure and arrangement between vaccinia virus and orf virus. *Virology*. 195, 175-184

Fleming, S.B., Lyttle, D.J., Sullivan, J.T., Mercer, A.A. and Robinson, A.J. (1995) Genomic analysis of a transposition-deletion variant of orf virus reveals a 3.3 kbp region of non-essential DNA *J Gen Virol.* 76 2969-2978

- Fraser, K. M., Hill, D. F., Mercer, A. A. and Robinson, A. J. (1990). Sequence analysis of the inverted terminal repetition in the genome of the parapoxvirus, orf virus. *Virology*. 176, 379-89.
- 5 Graham, F. L. and Van der Eb, A. J. (1973). A new technique for the assay of infectivity of human adenovirus type 5 DNA. *Virology* 52, 456-467.
- Johnson, K.S., Harrison, G.B.L., Lightowlers, M.W., O'Hoy, K.L., Cougle, W.G.,
 Dempster, R.P., Lawrence, S.B., Vinton, J.G., Heath, D.D., and Rickard, M.D. (1989)
 Vaccination against ovine cysticercosis using a defined recombinant antigen. *Nature* 338 585-587.
- Lyttle, D.J., Fraser, K.M., Fleming, S.B., Mercer, A.A. and Robinson, A.J. (1993) Homologs of vascular endothelial growth factor are encoded by the poxvirus orf virus. J. Virol. 68 84-92
 - McGeoch, D. J. (1990). Protein sequence comparisons show that the 'pseudoproteases' encoded by poxviruses and certain retroviruses belong to the deoxyuridine triphosphatase family. *Nucleic Acids Res.* 18, 4105-10.
 - Mead, D. A., Szczesna-Skorupa, E. and Kemper, B. (1986). Single- stranded DNA "blue" T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. *Protein Eng.* 1, 67-74.

20

- Melton, D. A., P.A., K., Rebagliati, M. R., Maniatis, T., Zinn, K. and Green, M. R. (1984). Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing bacteriophage SP6 promoter. *Nucleic Acids Res.* 12, 7035-7056.
- Mercer, A. A., Fraser, K., Barns, G. and Robinson, A. J. (1987). The structure and cloning of orf virus DNA. *Virology*. 157, 1-12.
 - Mercer, A. A., Fraser, K. M., Stockwell, P. A. and Robinson, A. J. (1989). A homologue of retroviral pseudoproteases in the parapoxvirus, orf virus. *Virology.* 172, 665-8.
 - Merchlinsky, M. and Moss, B. (1989). Resolution of vaccinia virus DNA concatemer junctions requires late-gene expression. *J Virol.* 63, 1595-603.

- Miller, J. H. (1972). "Experiments in Molecular Genetics." Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Moss, B. (1990). Regulation of vaccinia virus transcription. *Annu Rev Biochem.* **59**, 661-5 **88**.
 - Moss, B. (1990). "Poxviridae and their replication." in Virology, B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath and B. Roizman, 2nd. Raven Press, New York, 2079-2111.

10

- Moyer, R. W. and Graves, R. L. (1981). The mechanism of cytoplasmic orthopoxvirus DNA replication. *Cell.* 27, 391-401.
- Mulligan, R. C. and Berg, P. (1980). Expression of a bacterial gene in mammalian cells. *Science.* 209, 1422-1427.
 - Mulligan, R. C. and Berg, P. (1981). Selection for animal cells that express the Escherichia coli gene coding for xanthine-guanine phosphoribosyl transferase. *Proc Natl Acad Sci USA*. 78, 2072-2076.

- Naase, M., Nicholson, B. H., Fraser, K. M., Mercer, A. A. and Robinson, A. J. (1991). An orf virus sequence showing homology to the fusion protein gene of vaccinia virus. *J Gen Virol.* 72, 1177-1181.
- 25 Robinson, A. J., Ellis, G. and Balassu, T. (1982). The genome of orf virus: restriction endonuclease analysis of viral DNA isolated from lesions of orf in sheep. *Arch Virol.* 71, 43-55.
- Robinson, A. J., Barns, G., Fraser, K., Carpenter, E. and Mercer, A. A. (1987).

 Conservation and variation in orf virus genomes. *Virology*. 157, 13-23.
 - Robinson, A.J. and Balassu, T.C. (1981) Contagious pustular dermatitis (orf). Vet Bull 51, 771-761
- Robinson, A.J. and Lyttle, D.J. (1992) "Parapoxviruses: their biology and potential as recombinant vaccines." in Recombinant Poxviruses, Chapter 9, 306-317 eds M.Binns and G. Smith CRC Press, (1992), Boca Raton.

- 32 -

- Sambrook, J. Fritsch, E. F. and Maniatis, T., Molecular Cloning: A Laboratory Manual (Second Edition), Cold Spring Harbour Laboratory Press 1989.
- Sullivan, J.T., Fraser, K., Fleming, S.B., Robinson, A.J. and Mercer, A.A. (1995)
 Sequence and transcriptional analysis of an orf virus gene encoding ankyrin-like repeat sequences. *Virus Genes*, 9, 277-282
 - Viera, J. and Messing, J. (1982). The puc plasmids, an M13mp7 derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene.* 19, 259-268.

- Vogelstein, B. and Gillespie, D. (1979). Preparation and analytical purification of DNA from agarose. *Proc Natl Acad Sci USA*. 76, 615-619.
- Weinstock, G. M., Berman, M. L. and Silhavy, T. J. (1983). "Chimeric genetics with β-galactosidase in gene amplification and analysis." in Expression of Cloned Genes in Procaryotic and Eucaryotic Cells, T. S. Papas, M. Rosenberg and J. A. Chirikjian, Elsevier, New York, 27-64.

WO 97/37031 PCT/NZ97/00040 - 33 -

CLAIMS:

20

- 1. A parapoxvirus vector comprising a parapox virus containing exogenous DNA.
- 5 2. A vector as claimed in claim 1 wherein the parapox virus is orf virus.
 - 3. A vector as claimed in claim 1 or claim 2 wherein the exogenous DNA encodes at least one gene product.
- 10 4. A vector as claimed in claim 3 wherein one gene product encoded is an antigen capable of inducing an immune response.
 - 5. A vector as claimed in claim 4 wherein the antigen is selected from the group consisting of HIV envelope protein, herpes simplex virus glycoprotein, *Taenia ovis, Echinococcus granulosis* antigens, *Trichostronglylus* antigens, *Haemonchus* antigens, *Osteriagia* antigens and combinations thereof.
 - 6. A vector as claimed in claim 5 wherein the antigen is a *Taenia ovis* antigen selected from the group comprising *Taenia ovis* 45W, 16kd, 18kd antigens and combinations thereof.
 - 7. A vector as claimed in any one of claims 3 to 6 wherein the exogenous DNA further encodes at least one product which is a biological effector molecule.
- 8. A vector as claimed in claim 7 wherein the biological effector molecule is selected from the group comprising γ interferon, IL-1, IL-2, IL-1β, IL-4, IL-5, IL-6, IL-12 and combinations thereof.
- 9. A vector as claimed in claim 8 wherein the biological effector molecule is selected from the group comprising IL-1, IL-2, IL-12 and combinations thereof.
 - 10. A vector as claimed in any one of claims 3 to 9 wherein the exogenous DNA further encodes at least one peptide moiety expressed as a hybrid or chimeric protein with a native virus protein.
 - 11. A vector as claimed in any one of claims 1 to 10 wherein the exogenous DNA is incorporated in one or more non-essential regions of the virus genome.

12. A vector as claimed in claim 11 wherein the non-essential regions are selected from the non-essential regions identified in accompanying Figures 2, 3, 5 and 7.

- 34 -

- 13. A vector as claimed in claim 11 or claim 12 wherein the non-essential region is from nucleic acids 11 to 16 in the sequence of Figure 5 or from nucleic acids 2226 to 2286 in the sequence of Figure 9.
 - 14. A vector as claimed in any one of claims 1 to 13 wherein the exogenous DNA is under the control of a poxvirus promoter.
 - 15. A vector as claimed in claim 14 wherein the poxvirus promoter is an orf virus promoter.
- 16. A vector as claimed in claim 15 wherein the orf virus promoter is selected from the group consisting of E1L, F1L and F3L as set forth in Figure 10.

10

25

- 17. A vector as claimed in any one of claims 3 to 16 wherein the exogenous DNA further encodes a reporter gene.
- 20 18. A vector as claimed in any one of claims 3 to 17 wherein the exogenous DNA further encodes a selectable marker.
 - 19. A fragment or variant of a vector as claimed in any one of claims 4 to 18 having equivalent immunological activity thereto.
 - 20. A vaccine comprising a viral vector according to any one of claims 1 to 18 or a fragment or variant thereof as claimed in claim 19.
- 21. A vaccine as claimed in claim 20 which further comprises a pharmaceutically acceptable carrier and/or adjuvant therefor.
 - 22. A host cell incorporating a vector as claimed in any one of claims 1 to 18.
 - 23. A host cell according to claim 22 which is a eukaryotic cell.
 - 24. A host cell according to claim 22 or claim 23 which is a bovine testis cell or an ovine testis cell.

- 35 -

25. A method for producing recombinant parapoxvirus vectors comprising transfecting a vector of any one of claims 1 to 18 into a selected host cell infected with orf virus; selecting a recombinant virus; and optionally purifying the selected virus.

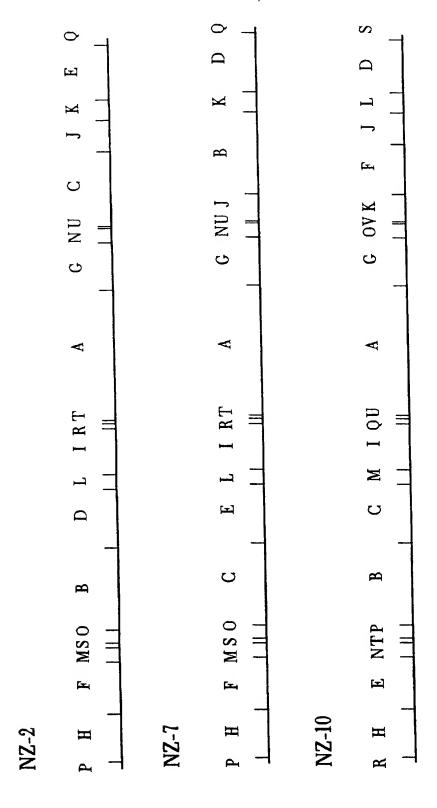


Figure 1. KpnI Maps of Orf Virus Genomes.

Figure 2. Nucleotide sequence of part of the KpnI E fragment of orf virus strain NZ-2.

7	1 21	31	1 41	51	19	17	81		
TCCAC	gocrircae econocier con conserva de cercande reseason en consensado en escante escante escante en consensados e	CGGAGCTCAC	GGTCTCGTGC	TCCCCCCCCA	CCTGCGACCG	crrccccrc	cecererect	CGCCGCACCC	CATCGAGCAC
111	1 121	131	141	151	161	171	181	1 191	,
ວຍວວວ	creecede regreeces caacerere	CGACGTCTTC	TCCCGCTGGA	recegerada Tanargecede egegoacece ecegacece ececaetere ergageceae ececegeges	CGCGGACGCC	CCCGACGCCG	CCGCACTCTC	CTGAGCCCAC	ອວອອວອວວວອຸ
211	1 221	231	241	1 251	261	271	1 281	1 291	
GCTCGC	cessecrese teraceaese crincinese escrincinse secosorisse escoceces secosocer coseceres coccineces sinosorises	CTTCCTCGCG	CGCTTCCTGC	GCCGCTGGC	ອວອວອວອວອວ	GCGCCGGCCT	cecceccre	cecerecee	Greegraces
311	1 321	331	341	151	361	371	1 381	391	
ອນນອອນ	receceace croceane recenecra tegrecran recerecene deceneces cesececer cecethece receceace recesses	TGCGAGCTAG	TGGTGCTGAA	ccccrcccac	GCGGACGCGG	cceccececr	cecectaece	receedade	rcaccaagac
411	1 421	1 431	441	451	461	471	481	1 491	
GCGGAG		 CGGACAAGCT	caccarcaca	cecesteses etesasetes sestesace caacaeece dasetsace esaceese etesasease	GCGTGGACCC	CGAGCACCCG	GAGCTGACGC	CGGACCCCGC	crececaeec
511	1 521	531	541	195	561	571	581	1 591	
GCGCAC	GAGAGGGCAC TCGCACAGAA CATCGACATC CAGACGCTGG ACCTGGGCGA CTGCGGAGAC CCCAAAGGCC GCCGACTGCG CGTGGCGCTG GTGAACAGCG	CATCGACATC	CAGACGCTGG	ACCTGGGCGA	CTGCGGAGAC	CCCAAAGGCC	GCCGACTGCG	careceere	GTGAACAGCG
611	1 621	631	641	651	661	671	681	1 691	
אכפכפפכ	gecacecese cecaaacte ecectesese ecetesesaa cececteace esceseste ececesese deacesect ecesasses ecaceces	GCGCTCGCGC	GCGTGGCGAC	CGCGCTGACG	Secondre	CCGCGAGCCG	GCACGGCCTC	פכפעפפפתפ	פטכפככפככ
711	1 721	167	741	751	761	771	781	1 791	
ACGCTG	Greakcecre crecreaces resecceder akcestecre secstecres cestificaer ecreceses	TGGCCGCGGT	GACGGTGCTC (gecerected (SGGTTTCACT (scrececec (GCGCTGCGGG TACGCTACCG	TACGCTACCG	crrcccacec
811	1 821	831	841	851	861	VEC	VEGF-2 →	881	891
ລອລອລລ	CCGGCCGCGC TGCGCGCGTA GCGCGCGAAA ATGTAAATTA TAACGCCCCAA CTTTTAAGGG TGAGGCGCCA TGAAGTTGCT	GCCGCGCAAA	 	TAACGCCCAA (TTTTAAGGG	rgagggggga		CGTCGGCATA	CTAGTAGCCG
911	1 921	931	941	951	961	971		991	
CTTGCA	TGTGCTTGCA CCAGTATCTG CTGAACGCGG ACAGCAACAC GAAAGGATGG TCCGAAGTGC TGAAAGGCAG CGAGTGCAAG CCTAGGCCGA TTGTTGTTCC	CTGAACGCGG	ACAGCAACAC (GAAAGGATGG	rccgaagrgc	rgaaaggcag (CGAGTGCAAG	CTAGGCCGA	TGTTGTTCC
101	1021	1021 1031 1041 1051 1061 1071 1081	1041	1051	1061	1071	1081	1601	
AGCGAG	TGTANGCGAG ACGCACCCAG AGCTGACTTC TCAGGGGTTC AACCCGCCGT GTGTCACGTT GATGCGATGC	ACGCACCCAG AGCTGACTTC TCAGGGGTTC AACCGGCCGT GTGTCACGTT GATGGGATG	TCAGGGGTTC)	AACCCGCCGT C	TGTCACGTT C	SATGCGATGC C	SCCGGGTGCT (GCAACGACGA	GAGCTTGGAA
:::: :::::		1131	1141	1151	1161	11711	1181	1191	
TCCCCA	recetececa eggaagaagt aaacgtgaeg atggaactee teggggeget gggeteeggt agtaacggg tecaacgtet sacties	AAACGTGACG 7	ATGGAACTCC 7	GCGTCCCCA CGGAAGAAGT AAACGTGACG ATGGAACTCC TGGGGGCGTC GGGCTCCGGT AGTAACGGGA TGCAACGTCT GAGCTTCGTA GAGCATAAGA	GGCTCCGGT A	GTAACGGGA 1	GCAACGTCF	SAGCTTCGTA (GAGCATAAGA

Fig 2. 1



Fig 2.2

Nucleotide sequence of part of the KpnI D fragment of orf virus strain NZ-7. Figure 3.

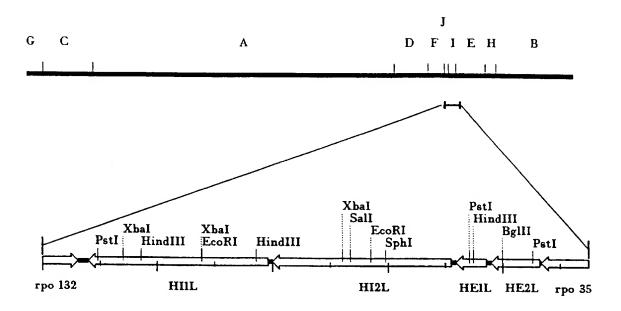
	CGAGCAC		໑ຉຉ໑ຉ໑໑		GCGGTGC		GAAACGC		CGGGCGA		CAGCGGC		ccccer		CAAAGTC		ACGTCAT		TGATTCACCT		AGCACTAACC	ATACAACTGT
16	ccc cAT	191	ວຍລຸ ລວ <u>ອ</u>	291	gre egr	391	cre ece	491	ccr cce	591	GGT GAA	691	3GC ACG	191	SAT ACTO	891	TT AAA	991	raa TGAT	1091	AA AGCA	AA ATAC
~ 4 ~	r ೧೯୯୯ ೧೯		AGCCCAC		cerecec		ಕ್ರಾಂತ್ರವಿಕ್ಕಾ		GACCCCG		resecte		GGAGGGC		GGTTTAG		FAACATT		CTCAGAGTAA		ATATCCAGAA :::::::::::::::::::::::::::::::::	GAAACAAGAA
71 81	 GEGCTGCTCT GEGAGCTCAC GETCTCGTGC TCGCGCGGGA CCTGCGACCG CTTCCGGCTG CGCGTGTCCT CGCGGCACCC CATCGAGCAC	1 181	resticacce carcitate receserasa raaatseese recasacsee eccaacsee lactereers ascelacsee escisesees	1 281	יוורכובכיסים פסווססכיסים פיסכיסיסים כיססכיויסים לכסכיוסים למוסכיסים למוסכיסים ב	381	recearded dacecedere dececerce decreecere dececera deceanance	1 481	caractradac Gradaccece Agerecedaa Gergacgece Gacceceer Gegeggega	1 581	chaggesact gesecsaces changeses deactsess tosesetses sancheses	1 681	canacrecec ecrescecec erescence escrences ececerece ecanoces hoseceres éanseces hoseces	1 781	GCTAAGAATA CGGTTTAGAT ACTCAAAGTC	1 881	CITAGAGIGT AACTITIGAGT AAAAAAIGTA AATACTAACG CCAAAATTIC GATAGITGIT AAGCAATATA TAACAITITT AAAACGICAT	1 981		1081	GTTGTTTATT TGGGAGAAGA ÅTATCCAGAA ÅGCACTAACC	TACAGCGGTT GAAACAAGAA ATACAACTGT
61 7	CTTCCGGCTG	1 171	CCCGACGCCG	1 271	CCGGCCTCGG	371	GGCGCGCTCG	1 471	AGCACCCGGA	1 571	CAAAGGCCGC	671	GCGAGCCGGC	171	receccecec	871	SATAGTTGTT	176	 TAATTTGCCA GAATGCGTGT	. 1071	GTTGTTTATT	GTCAAATATG 1
	creceacce	1 161	TGCAGACGCC	1 261	ودودودوووو	1 361	GACGCCGGCC	461	GTGGACCCCG	1 561	GCGCGACCC	661	осесетессс	761	ATTTCGCTGC	861	CAAAATTTC	961	ratgratgra	1061		ACGGTGACG C
1 21	TCGCGCGGGA	151	 TAAATGCCGC	251	GGCTGGCCGC	351	CTGCCACGCG	451	CGAGCTAGGC	551	CTAGGCGACT	651	CGCTGACGCG	751	Greerecca	851	NATACTAACG (951	CATTGTTAA	1051	TTGTAAACC 7	derrecrer J
7	 Gercresec	141	TCCCGCTGGA	241	TTCCTGCGCC	341	TGCTGAACCG	441	ccercecece	541	GACGCTGGAC	641	GTGGCGACCG	741	cogrectede	841	AAAAATGTA 1	941	GTTGTTGTT	1041	GACAAAGTG GTTGTAAACC TAGAGATACT	ACGATGCAG 1
1 3	CGGAGCTCAC	131	 cgacgrgttc	231	ACGACGTCTT CCTCGCGCGC	331	GCGGAACTGC GAGCTGGTGG TGCTGAACCG	431	GCCGCGCGC GACAAGCTCG CCGTCGCGCG	531	GCACAGAACA TCGACATCCA GACGCTGGAC	63]	GCTCGCGCGC	731		831	AACTTTGAGT	931	CTACGITACA AGIIGIII GCALIGIIAA IAIGIATGIA	1031	VICACTIGGAT GCGTACACTA GACAAAAGTG GTTGTAAACC TAGAGATACT	 Granctetta Arcgatecag Tegitecist Arcestarg
1 2]	 GCGCTGCTCT	121	 recrececes	1 221	 ACGACGTCTT	321	GCGGAACTGC	421	9090909009	521	GCACAGAACA	621	CGAACTGCGC	121	 GCTGGCGGTG	821	CTTAGAGTGT	921	AAGTTAACAG	1021	ATGACTGGAT GCGTACACTA	TCCCCGGTGC GTAACTGTTA AACGATGCAG TGGTTGCTGT AACGGTGACG GTCAAATATG
1	 GGGCTTCCAC	11.	רדכפכפכפפכ	1 211	 GGCTCGCTGT	311	GCGGTCGCCT	411	 TGGCGGAGCT	511	GAGCGCGCTC	611	CACGCGGCCG	711	 	811	 TATCCAGACA (911	FF 7 -	1011	ccrrcaacca 2	 TACAATATAA 1 ::::::::::
		101		201	_	301		401	_	501		601	`	701		801	_•	901	_~~	1001	1101	· `

Fig 3.

1201	1211	11 122	21 123	31 124	ti 1251	126	1271	128	81 129	-
	AACAGTTTCA		reperagence	GTCTGGTACT	GICTGGTACT ANTAGTGGTG TATCTACTAA CCTTCANAGA ATAAGTGTTA	TATCTACTAA	CCTTCAAAGA	ATAAGTGTTA	CAGAACACAC	AAAGTGCGAT
1301	1311	1321	11111111111111111111111111111111111111	1341	1321 1331 1341 1351 1371 1381 1391	1361	51 1371 	71 1381 	81 139 	- - - - -
~ ~	TGTATTGGTA G	GAACAACGAC	AACACCTACG	ACCACTAGGG	saacaacgac aacacctacg accactaggg aacctagacg ataactaata acaaaaatg titatititg	ATAACTAATA	ACAAAAATG	TTTATTTTG	TAAATACTTA	ATTATTACAC
1401	1411		1431	11 1441	1451	1 1461	1471	148	31 149	-
_~	ACTITACAAT	AATCTCAAAA	ATAAATTGCG	TGCCCGGACG	ACTITACAAT ANTCICAAAA ATAAATIGGG IGCCGGGACG GCIGCAGCIG GIGACGCIGC IGIGICACAC ACIGCGIAII CGAIICAAGI	GTGACGCTGC	TGTGTCACAC	ACTGCGTATT	CGATTCAAGT	TCACTAACGC
1501	1511	1 152	1 1531	1 1541	1 1551	1 1561	1751 1571	1 1581	159	<u>-</u> -
_0	ACTAAACTA	CACTAAACTA GTTGTGCGTG	TCCGAGTGTT	AACCGTACGT	TCCGAGTGTT AACCGTACGT CAAACTAACA TCTTACCTGT CCGTGACAAG AACTAAAACT	TCTTACCTGT	CCGTGACAAG	AACTAAAACT	TGAACCACAT ATTTTTAAAG	ATTTTAAAG
1601	1611	1 162	1 163	1 1641	1 1651	1 1661	1 1671	1 1691	1691	
-₽	ATATTTAAC	TATATTTAAC AAAATCACTC	ACACTCACAC		ANTCATAAAC ACCACAACCA CAACCAAACA CGCATGAGAA TTAATATTCT TACTTATCCG TAACACTCTA	CAACCAAACA	CGCATGAGAA	TTAATATTCT	TACTTATCCG	TAACACTCTA
1701	1711	1 172	1 1731	1741	1751	1 1761	1771	1 1781	1 179	
_₽	TGCTGTACAT C	CAACGCATCA	GAGCAGTCTG	AGTCTGACTA	anggerica gageagicte agictereta atggeggeaa acgggaacge aggeggaea taateactea gaateteege ageaacege	ACGGGAACGC	AGGCGCGACA	TAATCACTGA	GAATCTCCGC	AGCAACCGCT
1801	181	182	1 183	1 1841	1 1851	1 1861	1 1871	1 1881	1 1891	
_ପ	AAGGACATC	TCTAGCGCTA	CAAGGACAIC ICIAGGGCIA ACGGCIGIT GICAIICCCC	Grcatrecee	CGTGTGTTCA TCTCACACGA CATTGTGACC GTCGCAAAGC ACACATTCAA AGTGCCGCAT	TCTCACACGA	CATTGTGACC	GTCGCAAAGC	ACACATTCAA	AGTGCCGCAT
1901	1161	1921	1931	1941	1951	1961	1 1971	1 1981	1 199	
_ច	GTGGAAGAAT TO		GACACACACC	ATAATTAAAC	ACCOTCGA GACACACAC ATAATTAAAC AAGATCAGTG CATAAGAGAG ATTAGCATTC TACAGCACAC CACGTGCGAA TACGGACCTC	CATAAGAGAG	ATTAGCATTC	TACAGCACAC	CACGTGCGAA	TACGGACCTC
2001	2011	2021	2031	2041	2051	2061	2071	2081	209	
្ទ	GTAATTGTTT AG	AGACTAGAAC	Accretegre	TAAACAACAT	actagaac acceptegec taaacaacat geccgaetet agaacagage teatgacgca taigeaactg igetetetat geagaagtea	AGAACAGAGT	TTATGACGCA	TATGTAACTG	TGTTCTTTAT (GTAGAAGTTA
2101	2111	2121	2131	2141	2151	2161	1 2171	2181	2191	
ĭ	TCTTTTATGT CA	CACTCCCTTG	TCTTAGATGA	GTTATACATG	CTCCCTTG TCTTAGATGA GTTATACATG ACATGATGTA TGTGTCGCCC	rererecec	202002000	GGGCGCTCG	פפפכפכדכם פכפכפכפכם הכרבכככככ	recrececec
2201	2211	2221	2231	2241	2251	2261	1722	2281	1 2291	
ဗ) <u> </u>	ceereeceec	GGCTGGCGCG (3030T-8086 (GOEGGGCCCG CGGTGGCGGC GGCTGGCGCGCGCGGGGGCG GCGCGGGGT AGCGGCCGC CCGCCGGC GCCGCGCA GCCTTGCCC	sceeceeeer .	AGCGGCCCGC (2000222002	cccccccc	SCCTTGCCC
2301	2311	2321	2331	2341	2351	2361	2371	2381	2391	
ဗ္ဗ	GACCAGGC (SCCACGGAGC 1	AAAGTGAAAA	AGGACCGCCT }	AGCAGTCGAG A	ACCCTCCCGC (ceckeceses !	ACACCCCACA C	ccecerrec	ACCCGCCAGA
2401	2411	2421	2431	2441	2411 2421 2431 2441 2451 2461 2471 2481	2461	2471	2481		
ဗ္ဂ	CCAACACC	ACAGCCAACA	AGCATGCACC (יכדכפככפכפ	GECCAACACE ACAGCCAACA AGCATGCACE COTCGCGGG CAGGCTGCTC GGCGCGCTCG CGTGGTGGCG CTGGGTTCC TCCTCGGCGG	sececerree (cerecreece c	יוספפכדוככ י	rccrcacaa	

6/34

FIGURE 4. HindIII Map of Orf virus NZ-2 showing the location and orientation of the reading frames for the putative genes, rpo132, (H)I1L, (H)I2L, (H)E1L, (H)E2L, and (H)E3L (rpo35).

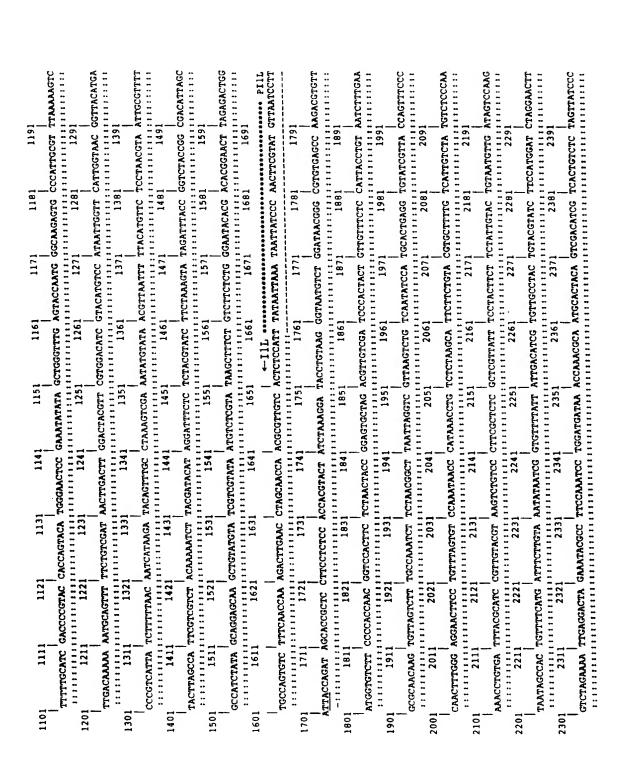


(4750 bps)

Figure 5. Nucleotide sequence of genes HIIL, HI2L, HEIL and HE2L from orf virus strain NZ-2 showing potential insertion sites.

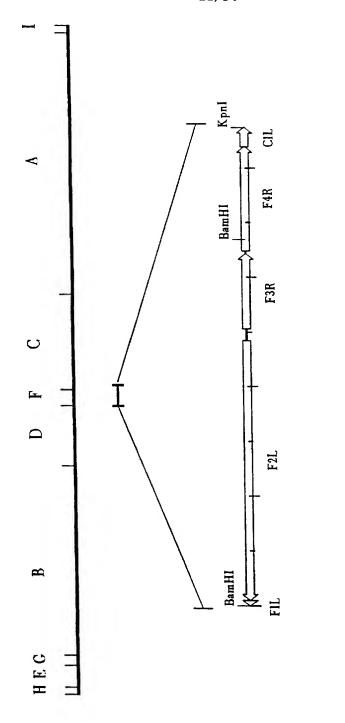
-	11 21		31, 41	1 51	61	17.	8 -	1 91	
 Gac <u>tga</u> tgcg	-	9090000095 	ACTTAGCTTA	AAACGCGCGG CGGCGCCGCG ACTTAGCTTA TCTCGACTGA TGCGAACGCG CGACCTCTCG CGACTTTCTA GCTTCTAGTGCTAC	receaacece c	GACCICICG	CGACTTTCTA	GCTTCTCAGA	CTGATGCTAC
111	121	131	1 141	151	161	171	181	191	
CATATCGCGG (CCACCACCAG of	GGCTTCTCGC	cenerade cencencea decrreted cereacidad decadecida errecandes decracada herrecedes ceceatede cereacidad con central con transfer con tr	GCGGGGCTGG C	CTGCGACGCG C	CGCTGCAGTA C	GCTGCGCGCG (1111111111111111111111111111111	CCCCAGTCGC
CGCGCACGTG		AGGCTCCCGT (CCAGCGCGTG C	CGCCGGGGGC AGGCTCCCGT CCAGCGCGTC CCGCGTCACC TCGGCGCCGG GCCGGCGGCA CGTGTGCACG TCCGTCTTGT TGGACACGAG 11111111111111111111111111111	TCGGCGCCGG C	GCCGCCGCA C	CGTGTGCACG 1	TCCGTCTTGT 11111111111111111111111111111	TGGAGACGAG
CACCGCGTAC		TCTCTATGTG	ATGCTCCAAG	TOCCGCAFGG TCTCTAFGFG ATGCTCCAAG TGCTTGCCCG CCATCCGGTT GGACTCGCAG CACGTTTTG CTFCGCTAA GGTTTTTCT 11111111111111111111111111111	CCATCCGGTT C	GGACTCGCAG C	CACGITITIG C	CTTCGGCTAA (3GTTTTTCT
AGAGGGGATA		CACGCGCTCG	GGCAGGACGC	GTAGCTTATC CACGCGCTCG GGCAGGACGC ACGCGGAGCC GTCGAACCT ACTTTGAACG GGGTCACCTT GATGTTCCCG TCGTAGCGGT 11:11111111111111111111111111111111	GTCGAACCCT A	ACTTTGAACG (GGGTCACCTT G	GATGTTCCCG 1	rcgragcggr
CCCACAGCAT		GTTGTACCGT	CGGGGTCTGG	CCTGAGGTAG GITGTACCGF CGGGGTCTGG GICTGTCCAC ACTCTAAGGT TITCGGTACA GCGGCCGTCG TACGTAAGAC GGTCTCTACG (111111111111111111111111111111111111	ACTCTAAGCT 7	ITTCGCTACA G	GCGCCGTCG T	TACGTAAGAC G	3GTCTCTACG
 CTCGTAGTAG T	— ~~	TGTTGTTGGG	Gretcearge :	TTTCTGCTTA TGTTGTTGGG GTCTCCATGC TCGTAGTAGT ATANATCGTA CGCGCCTGGC TTTTTAAGT CGTTTTCGTC GTTGCTGACG (111111111111111111111111111111111111	ATAAATCGTA (cececerede 771	TTTTTAAGT C	CGTTTTCGTC	STIGCTGACG
 TGTATCACGT (CGGGATAATA	GGATATCCTA	ACTGCACTAC 3	CGGGATAATA GGATATCCTA ACTCCACTAC AATCTATAGT ATTTGGTCTA GTAAGCTGTT CGAGATCACC TTGTTCATCA TGATCTACTG 11:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1:	ATTIGGICIA G	STAAGCTGTT C	CGAGATCACC T	TTGTTCATCA	GATCTACTG
ATTTGTACAC O	 GGCACCGTCG	TGTTCCGACG	GACGTATGAA	GGCACCGTCG TGTTCCGACG GACGTATGAA TATGTCCATG GTAAACGATG TACCCACTTT GGAAAACGTA TCCCATGCAG TAAAGCATAG	STAAACGATG 1	racccactrr c	GGAAACGTA	TCCCATGCAG	raargcatag 1:111111
 TCCGTCCATT A	 ATAACTCAG 	GAACACTCAT	AACAAATCGA	ATARACTCAG GAACACTCAT AACAAATCGA AATCTGTGAA GTTTTTCGAA CACCACTTTT ACATGGTCTT TGTCACGAAC ATCATTGCCG	STTTTCGAA C	ACCACTTT P	ACATGGTCTT 3	IGTCACGAAC	TCATTGCCG
TTTACTTCAG	ACATGAATTG	AAGGAACGCT	AAAGAGTTTC	ACATGAATTG AAGGAACGCT AAAGAGTTTC TTGTTTCTTC ATGAATCTTT CCATTATACG TCCATCCAGT TTCTAGAATT CTATATATGC	ATGAATCTTT C	CATTATACG 1	CCATTATACG TCCATCCAGT TTCTAGAATT CTATATATGC	rrcragaarr	TATATATGC

Fig 5.



401	2411	2421	2431	2441	2451	2461	2471	2481	2491	
_ `	ATCAACAACT	CCTTCTT	GATGCTCCAC	TGTTTTAAAG	Trancticate 3	TGTATATGTT G	GAGTGTTAAA 1111111111	AAAATATCCA 11111111111111111111111111111	CATTGAACGC	AGTICCATAT 1::::::::
2501	TTTGTTATT	TATCCCAC	CGTATAACAC	AAACCATCCA	TAATGAATTC O	CGGAACCGAT 7	ACTACAAAAT	 TAGTATCGAA : : : : : : : : : : : : : : : : : : :	CTTATCAAAC	GAAATGTTTA
2601 	2611 	CTCGCGGTTG TGCTTCGTAT TTTTTGTCAT ACACATCTGT CATGTATTGT ACAAAATCTA TAGCTCCTCT AGCATCGGGC ATGCTAACGT CTGGAAAATC CTCGCGGTTG TGCTTCGTAT TTTTTGTCAT ACACATCTGT CATGTATTGT ACAAAATCTA TAGCTCCTCT AGCATCGGGC ATGCTAACGT CTGGAAAATC CTCGCGGGTTG TGCTTCGTAT TTTTTGTCAT ACACATCTGT ACAAAATCTA TAGCTCCTCT AGCATCGGGC ATGCTAACGT CTGGAAAATCTA TAGCTCGTCT ACAAAATCTA TAGCTCGTCTAACGT CTGGAAAATCTA TAGCTCGTCTAACGT CTGGAAAATCTAACAACAACAACAACAACAACAACAACAACAAC	TTTTGTCAT	ACACATCTGT (CATGTATTGT A	ACAAATCTA 7	rageteetet	 AGCATCGGGC 	ATGCTAACGT	CTGGAAAATC 111111111111111111111111111111111
2801	ATTTTTTAAC :::::::::::	ATTITITAAC TGITCTAGIA CGAACTGITG AFFITGGICA TCTCTCCACC AGTACATTGG CAATCCTATC ACAGAGACT TAITITIATA ATANTITGCT ATTITITATA CAACTGITG ATTITITITITITITITITITITITITITITITITITIT	CGAACTGTTG A	ATTITGGTCA	TCTCTCCACC A	AGTACATIGG C	CAATCCTATC A	ACAAGAGACT	TATTTTATA 1::::::::::::::::::::::::::::	ATAATTTGCT
1,	ACAGAAGCTA	ACAGAAGCTA TATGCCACAT GAAATTATAG CAAAAATAAT CCATCTGTAT GTTTAAAACC GGTTTACTAG TCTGCTGAGA GTAGCTATCC ATGATAGTGT 	GAAATTATAG	CAAAAATAAT (CCATCTGTAT C	STITAAAACC G	3GTTTACTAG T	TCTGCTGAGA 1111111111111111111111111111111111	GTAGCTATCC :::::::::: 1 1 2991	ATGATAGTGT 1::1::::
	 	TTCCCTCGCC AATACTTCCT GACATTATTC TGTAAACAAC CATTAACAAA AATCTTCCTA CCGTTGTTAT GTTGTCAAAA GTTCTTATGT TTTGAGCACT :::::::::::::::::::::::::::::::::::	GACATTATTC GACATTATTC GACATTATTTTTTTTTTT	TGTAAACAAC 1111111111111111111111111111111	CATTAACAAA A	NATCTTCCTA C	CGTTGTTAT G	GTTGTCAAAA	GTTCTTATGT 111111111111111111111111111111111	TTTGAGCACT
101	TTCGAGTAAC	TTCGAGTAAC CATATGTTAT TTTCAAATAT ACTTTGTAA ACTGGATTTC TGTCCACATA ACTCTTTAAA AATAGATTTA CTGGAAGGCC GCTTGGGTTA ATTCGAAGT CATATGTTA ACTTGGATTA ACTGGATTA ACTTGGGTTA ACTGGATTA ACTTGGGTTA ACTGGATTA ACTTGGGTTA ACTTGGGTTA ACTGGGTTA ACTGGGTTA ACTTGGGTTA ACTTGGTTA ACTTGGTTA ACTTGGGTTA ACTTGGGTTA ACTTGGGTTA ACTTGGTTA A	TTTCAAATAT	ACTITIGIAA 11111111111	ACTGGATTTC 1	rgrccacata A	ACTCTTTAAA A	AATAGATTTA	CTGGAAGGCC (1111111111111111111111111111111111	GCTTGGGTTA
13201	rcrcccrgra	TCTCCCTGTA TAGGCGCCC TTGCTTTATG TATTCGCGTA ACAATCTCT TACAACTTTT CTAGTTCTTC TAGGTATACA TGACCTATTA TTCAAAGGCT	 TTGCTTTATG	TATTCGCGTA 7	ACAAATCTCT 1	racaactiti c	TAGTTCTTC T	TAGGTATACA	TGACCTATTA :::::::::::::::::::::::::::::::	TTCAAAGGCT
-	TGTCCAGTT	+-I2L ************************************	AACGITGIAA	 AGTCACTGAC '	TAGCTTCTCC	L	AATAATTAC	AGACGGCAAC	4-I2L BARTARTRA AGACGGCAAC ACHTATARTRA TAGACGGTTA T <u>CTR</u>	CTATATCT
3301	331	331 331 332 333 334 334 3351	3331	3341	3351	3361	3371	1866	1 3391	
J	CTGTATCCT	GCTGTATCCT GTCTGTACAT CTATTTTCT GTTGAGATCA AGAAGAGCTT TACGTAGACT CTCCAAGTGT CTTTCTAGTC TGTCTAACCG GTTACCTGTT	CTATITICI	GTTGAGATCA	AGAAGAGCTT 1	racgragacr C	TCCAAGTGT	CTTTCTAGTC	TGTCTAACCG	STIACCIGIT
3401	3411	3421	3431	3441	3451	3461	3471	3481	3491	
-	retergeAGC	TCTCTGCAGC AATCAGTTAT AGTTTTGTAA CTGTCTAACA AGCTTACGAG GCGCTCTTCC ACACTTTCTT TAGTTGGAGC TCACCGGG AATCAGTGGAGC AATCAGTGAA AGTTTTGTAA CTGTCTAACA AGCTTACGAG GCGCTCTTCC ACACTTTCTT TAGTTGGAGC TCACCGGG AATCAGTTATATA AGATTAGAA AGCTTACGAG AGCTTACGAG AATCAGTTAGAA AATCAGATATATATATATATATATATATATATATATATAT	AGTTTTGTAA	CTGTCTAACA	AGCTTACGAG (scererree A	ACACTITCTT	TAGTTGGAGC 1	TCAGCCCC 1	וארערזירניי
3501	3511	1 3521	1 3531	1 3541	3551	3551 3561 3571 3571	1/05 .		***************************************	* PEZL
	GGTTGAATT	TEGITGAATT GCCIGIATCA TCAGGCIGAG TCAATAGGIT TICTCCGICA ITTICATCCA TATIGAGICC AACGAACACA AACGAGIAAG IGITCCICIA	TCAGGCTGAG	TCAATAGGTT	Trerecerca 1	rrrcarcca T	PATTGAGTCC	AACGAACACA	AACGAGTAAG	NGTTCCT <u>CTA</u>

ttiaaagiat tgatititaga aaaagggaag cetegetege cegatitege egcaaacace egitgaacac écegaagtes étegeggeeg égaagatete cegegitege egegiteane tegeggaigi toregiagit etegiagae ececagaet ecaceceae daacateges ecececese eaggeceae 'erecegerae 'ectecrace 'regesanges 'eccnescens' nescestes 'eccangesan' cesanetes 'snesecesea 'estesses 'ettsansnes AUGCAGOGOC ACTICITIGOS CACGIOGAAS GOOTOGIOGIO TOGGGIOGAA CACGOGOCOG TOCACGOGOG GGCOGOCOGO CGIGOGGOGO AACTOCAGOG GCAGAGCGCG GACACCGCGT CCATCTTTTA TGTGCAGAAT TATTCGTTGG C



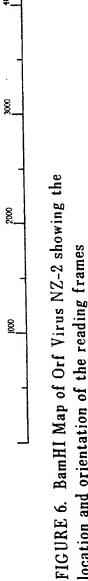


FIGURE 0. Damin Map of Old vills NE-E shows location and orientation of the reading frames for the putative genes FIL, F2L, F3R, F4R (topoisomerase) and C1L.

Figure 7. Nucleotide seguence of BamHI F and part of BamHI C from orf virus strain NZ-2 showing potential insertion sites.

	CCCCAGGA		ACATGTTGTA		GTTTGCGTAG		GCTTCATGT		cercrese		ACGGCGCGA		secesecar		STICACGAG		SCCATGAAC		reecceace		GCGTTGAT		TGAAGATG
1 91	AGTIATIAAA AITAATGAIA IAGAACICIT TIATGGGGGG IATGGAITGG GCIATCCAGI CCTIGACCGA GCCCACGAIG CCCGCCAGGA	191	crececeda A	1 291	ACATGGCCGG G	391	TGGTGTTTGT GACGAAGAGG CTCGCCAGCG AGATGATGAT TTTTTCTTC TCGATCTCGA TCTTGATGTG GTCCTCGAAG CGCTTCATGT	167		1 591	acticogogo acacogicog cotagogogo geogogogogo geatogogog egococoaco egotogogas egaaaaacto eacogogoga	691	corceceta caactecaec aggraginge gecigeege gereegeege argigitea geeggigeag circegeage ageeggeer	791	CAGCICCICG GCCAIGAIGG AGGIGIACAC CICGGICAGC AGCAICACGG IGICGAAGIC CICCIIGCCG CAGACGCGCG ICIICACGAG	891	GACTCGGTGA CCTCGACGCT GCGTCCTTG GTCTTCTTCG CGCTCCGCGA GGCCATGAAC	991	AGICCGCGCT GCTGIIGAGC ACGAIGACCA CGCGGAGGAI GAAGIIGAGG IICAGGGICI ICGCGGACIG GAACAGCICG GIGGCCGACG	1091	GCAGGAAGAA CACGCCGCGC TTGATCTCGG CCGCGAAGCG ACGTTCGTAC TCCTGCCGGC GCGCTTGAT	1191	TECACGGCCC AGGICTGCGT CTICAIGAIG GIGTCGAAGG ACAICACGAI GITGAAGAIG
1 81	CCTTGACCGA	1 181	GOCGANCTCC AGGTCCACGC GGTTCAGAGA GTCGCTGAAG TACACGAAGA CGTCGCTGTC CGGGAAGAAG CTGCGCGGA	1 281	TTGTGCGCGA CGTGCTCCGC GCTCAGGAGC GTCTCGTCGA AGGGGTACGG GTCGCTGAAG CGGAACACGT ACATGGCCGG	381	rcreargre	481	cecerceses	581	CGCTGCGAAG	681	sccerecae (781	recriece (881	rerrerres (981	CGCGGACTG G	1081	CGTTCGTAC T	1181	TGTCGAAGG A
1 71	GCTATCCAGT	1 171	cercecrere	1 271	GTCGCTGAAG	371	rcgarcrcga	471	Acreseses	1 571	CGCCCCCACG	671	ATGGTGTTCA (771	reregaagre c	871	secercerre e	971	TCAGCGTCT I	. 1071	CGCGAAGCG A	1171	TTCATGATG G
1 61	PF1L TATGGATTCG	161	TACACGAAGA	261	AGGGGTACGG	361	FTTTTTCTTC	461	ATGTCCTGGA	561	SCATGGGCCG	661	sereceecee .	761	GCATCACGG 1	861	CTCGACGCT 0	961	AAGTTGAGG T	1901	TGATCTCGG C	1161	Gercrecer c
1 51	TIATGGCGGC	151	GTCGCTGAAG	251	GTCTCGTCGA	351	AGATGATGAT	451	STCCGACATG	551	seceecerec (651	secrececer (751	TCGGTGAGC 1	851	ACTCGGTGA C	951	CGCCACGAT G	1051	ACGCCGCGC T	1151	TCACGGCCC A
1	TACAACTCTT TTATC	141	GGTTCAGAGA	241	GCTCAGCAGC	341	CTCGCCAGCG	441	GCACGCGCGA	541	CCTAGCGCGC	641	AGGTAGTTGC (741	AGGTGTACAC (841	ccccacccrc	941	CGATCACCA G	1041	CAGGAAGAA C	1141	GATGTTGTAC T
1 31	CTAAA ATTAATCATA TACAI	131	AGGTCCACGC	231	cerecrecec	331	GACGAAGAGG	431	ACGCGGATGA	531	ACACCGTCGG	631	GACTCCACC	731	SGCATGATGG	831	CGATAGAGAG C	931	CTGTTGAGC P	1031	GCGGAGAGCC G	1131	GAGCCGGTT G
1 21	AGTEATTAAA	121	GGCGAACTCC	221	TTGTGCGCGA	321	rgetettet	421	 GGTGTCGTGC	521	Accreeced	621	GTCCGCGTA	721	AGCTCCTCG	821	ACAGCCGTCG (921	 GTCCGCGCT	1001	GAGCAGGTTC G	1121	AAGTTCAGGA TGAGCCGGTT
:	←F1L ••••	111	ACAGGAAGAA	21.1	cccerrcacc	311	 TAGTACTTCA	411	 	511	, ၁၁၁၁၁၁၁၁ 	611	 BCCTCGCCCG	711	AGTGCGCCTC (811	 GAAGTGGTGC A	911	 Gagacgagga a	1011	 cerccacere 6	1111	 cgcgatgagg a
1		101	_~	201		301		401	_ ۲	501	_°	601		101	<	801	ల	901		1001	_ర	1101	_៩

ANGOGOTGGO TETECGAGAN GTAGOTGTAG GGOTCGOTGA GGAAGATGGA CTTGTTGGTO GCGGGCACON CCACGCCCGC GCGCGCGCG GACGCGTCGG TOTICAGGIC COGGANGING ANGECGEAGA INGEGECAGIA GOCCANDECG INCETERAAGI ACACGAACIC ENECACGAAC INGINGANET INGGEAAGIA GETCEACOTEC ACGEGEATEG CGACEGEGAG CEGGATETGG TECTEGEAGG GEGGEGACTE GAAGEGEAEC CETEGEEEC AGEEGGGGG CTEGGGEAEG ACCAGEGEGES TECECEARGE CEGGEGGAAC TIGGEGIUGE GUGGITIGAS CAGEGECEGES AAGAGGIUGE ÁGAGGIGECE GETUGAGAGS AACAUGIAUT ISTACAGCAG CEGGEGEGE INCEGEGGECA INGECGINERA GAAGGEGEGG CECERCINEG CGACEGEGGG CIGETECTEC GEAAGIIGI INGGGIAGAC ctistecesis secisesassa acaeetieti caestesass aastesessa teacsatsss saeseseses ecstesaset estaeatsaa caestasese 'AGGTTGAGCT TGCGCCGCGA GACCGGGATG CCGATGTGCC GACACAGGTA CGCGAACTCG AGGTACTTCT TCGAGAAGCG GATGCGGTCC AGGTTCTTGG Agacgracie cagcaigine cocaigina agaggaicic coccacgos gocicogos ceicgicaa agoggigos agaicgona i GACCACGGCT TCGCCGGTGG CGTCGTCGTG CACCAGCACG TTAACGCGCC GCTGCCGGAT GACCATGTCG AAGGTGTTGA AGAACATCTC GTACATGCTG cacgaagang gactnenter cegecgangaa greegignag gactnegnes ecenentese stegececans hadgegeges hentegecae sangenese 'recegagigi egicegegai gegetegeee 'acegagagae 'regeggigge 'stegicaege 'acetgetitei egaactigia 'ecegaigiag 'agaaiaiee 'AGAICAGGGI 'GGCGICGICG 'GCGICGGGGI 'ICIGCICCAI 'GGICGCGAAG 'AGCAGGCGGA 'IGICGICCIC 'CGIGAICGGG 'ICCACGIIGI ACAGGIIGAC

2401	1 2411	11 2421		31 2441	1 2451	1 2461	1 2471	1 2481	31 2491	-
	 aggatggact	r ccredaare	AGGATGGACT CCCTGGAATC CATTTAAGGA CGCCAAGGGC GCGCGAGCC GTCTCAAAAC TGAAATCGTA TAAACTCTA AAAATCGGT ATTGAAGTA	CGGCAAGGGC	GCGCGAGACC	GTCTCAAAAC	TGAAATCGTA	F3RTAAACTCTTA	TTAAGGA CGGCAAGGGC GCGCGAGACC GTCTCAAAAC TGAAATCGTA TAAACTCTTA AAAATCGGT ATTGAAGTA	ATTGAAAGT
2501	1 2511	11 25	2521 2531	11 2541	1 2551	1 2561	1 2571	1 2581	11 2591	-
•	**************************************	**************************************	F3R-> F3R->	F3R→ CATGTCTTCG	TGGCGACTCA	AAATGAGCAA	GTGTTCAGGT	 TCCAGCAGCG	 TCCAGACTCT	 CGAGGATCTG
10	261	11 26	21 2631	11 2641	1 2651	1 2661	1 2671	1 2681	11 2691	
_	GGTAATCGTC	 Treecrees	CGTAATOGIC TICGCICCGA GGCCITGGGC AACGAITGCC AAGAGCCCCG CGACGACCTC TICCCCAGCG GCGAGGAGIG ICTGGACAIC GACGGGCCCI	 Aacgattgcc) AAGAGCCCCG	CGACGACCTC	Trecenace	GCGAGGAGTG	TCTGGACATC	GACGGGCCCI
0.1	172	11 27	21 273	1 2741	1 2751	1 2761	1,772	1 2781	11 2791	
_	 GCCCTTGCGA	 TGAGGCGGAG		ACCAGGAGCA	 GTTGCCCGTG	CCCGAAACCG	I TGCCCGAACC	GCCGGCCAAG	ACTCCTAAGC	GCCGACCAGT
0.1	281	11 2821	21 2831	1 2841	1 2851	1 2861	1 2871	1 2881	11 2891	
	 GAAGAAGGAT AAG	 AAGGCAGATA	 Gecropia aggergatar gercargeteg Accagaggeg Canagarace Gecettegg Acgacargga Torgaggete Argagerarce	GGACAAGTCG	ACCAGAGGCG	CAAAGAAACC	Grecerres	ACGACAAGGA	rgacgagete	AAGAGCAACG
01	291	11 297	21 293	1 2941	1 2951	1 2961	1 2971	1 2981	11 2991	e-4
	ACGTCGACAA	 CAACGAAGAG	ACGECGACAA CAACGAAGAG TCCGGCGACA CAGACGCGG CGAGCGCCCG AAGCCCCAGG GACAEGGACA ACGEGGAGGA AAEGACGAC ECGACCECA	CAGACGGCGG	CGAGCGCCCG	AAGCCCCAGC	GACATCGACA	ACGTGGACGA	AATGGACGAC	rccacctca
~	301	.1 3021	21 3031	1 3041	1 3051	1 3061	3071	1 3081	3091	-
	 TGTGGCGTT CTC	 crccaccatc	CCACCAIC CICGCAGACT ICAAGGACCI IACCCAACGA GIGAAAGCIC TITCGICCGI GCICACGGAC GIGCAGGCGG CCGGCAIACG	TCAAGGACCT	TACCCAACGA	GTGAAAGCTC	rrrcerccer	GCTCACGGAC	GTGCAGGCGG	CCGCCATACG
0.1	311	i 312	21 313	1 314	1 3151	3161	3171	1 3181 1 PF4R	11 3191 R ************************************	1
	CAGGAGCTTC	 TCGACGCTCG	CAGGAGCTTC TCGACGCTCG GCAAGGCTCT GACGGAGGCG GCCCACATCG CCAACACGGG ATCTAAGCCA GTCACTGCGC CTCGCAAGAA GAAGGCCGCC	GACGGAGGCG	GCCCACATCG	CCAACACCGG	ATCTAAGCCA	Grcacrecec	CTCGCAAGAA	GAAGGCCGCC
10	3201 3211	1 3221	3231	1 3241	1 3251	1 3261	3271	1 3281	1 3291	н.
<u>.</u> 0	GCCTGCAAGA AGI	AGTAGGCGCA	GCCTGCAAGA AGIAGGCGCA CTANAIAGCG AGGCTCGGTA TGCGGGCGCT GCACCTGTCA GACGGCAAAC TTTTTTTGA CAAGGAGCTG ACGCAGCCGG	AGGCTCGGTA	F4R-	 GCACCTGTCA C	ACGCCAAAC	 TTTTTTTGA	CAAGGAGCTG	 AcgcAgccgg
0.1	331	1 332	ii 333	1 334]	1325	3361	3371	3381	1 3391	
	TCCCCGACGA	CAACCCCGCG	 recedencea careceges taccetste tiscgargat eeggateera eegcaectet eggatstegt eststacsas eaggaeetes agtetsegea	TIGCGAAGAT	CCGGATCCCA	CCGCACCTCT	CGGATGTGGT	CGTGTACGAG	CAGGACCTCG	AGTCTGCGCA
0.1	341	1 342	343	344	345	3461	3471	1 3481	3491	
~	GCAGGGCCTC	 Atcttcgtcg		CAAGGGCCGA	AAGCAGTACT	rcraceece (CGGACACGTG	GAGCGGCGCA	ceecearce	CAACGCCGTG
	3501 3511	1 3521	1 3531	1 3541	3551	3561	3571	3581	1 3591	
-	Trestecees	 TGCACCGCGT		ATAAACGCCT	TCATCGACGA	CCACCTCGCC	rccggcAGCG	AGGCCGAGGC	GCAGATGGCC	sccrrccrsc

	CGA		CTG		TCA		c,TG		360	1	908		990		
	Accresce		၂၀၁၀၀၁၀၁		ATCCGCG		CGCTCAT		CAGGGAC	5	GATGAGG		cecrrccc		
1 3691	AGCTTUTTE ATCCGCGTCG GCAAGACGCG CTACGAGCGC GAGAGCGGCA CCGTGGGCAT GCTCACGCTG CGCAACAAGC ACCTCGCCGA	1 3791	Ageggererr	1 3891	Accentegge	1 3991	rccgrgaagr	1 4091	regageregr	1 4191	PCIR GATCGTGGG GACTATGTAA ATAACTCTGA GCAGGTAAAT GGA <u>IGA</u> GGG	4291	ccccaaccc		
1 3681	GCTCACGCTG	3781	CGCGAGGGGC	1 3881	cerrearged	1 3981	GCGGGTCCC	1 4081	Acceceerec	1 4181	ATAACTCTGA	428	cccasccc		.K
1 3671	CCGTGGGCAT	1778	GTTTGCCGTG	3871	cccarctaca	1 3971	GCTCGCTGGA	1 4071	CTACATGGCC	1 4171	GACTATGTAA	4271	GCGCAGCTGC	4371	cerceceera (
1 3661	GAGAGCGGCA	1 3761	TCGCGCACGA	3861	GAGCGAGCGC	1 3961	TCCAACGTGC	1 4061	CGCGCAGCGC	1 4161	CATCGTCGTG	4261	cerererere	1964	GGGACGTGG
3651	CTACGAGCGC	3751	AAGGACCGAG	3851	TCGACCGGCT	3951	CAACTTCTGG	4051	CCCTCGATCT	4151	ACTTCGTGGA	4251	rggAcgrggc	4351	Treecege
1 3641	GCAAGACGCG	3741	crrceredec	3841	AGGCTGCTGT	3941	CCTTCCTGTA	4041	GGGCACACG	4141	PC18 ACGCTCGACG	4241	רדככפפככפכ	4341	regactitica c
1 3631	Arccecerce	3731	receestese	3831	CGCGCCCGAC	3931	GTGAACTACA	4031	 ccgagacggt	4131	GCCACCGAC	4231	CGTGGACGGG	4331	AGGCCTTCC 7
3621			GGTGAGGAGA	3821	GGGACCCGGG	3921	CACCTACGGC	4021	CGGCAGACCG	4121	 ACAGAGTCGC	4221	 - ceecececer	4321	arcerceec (
3611	.ATG	3711	~ ~	3811	 cgregecier eggaceeggg egeeceegae aggeigeigi icgaceeger aggegaege egegigiaea écticatoso àcgeilegge àteegeetea	3911	AGRICCIG <mark>CG CACCIACGGC GIGAACIACA CCITCCIGIA CAACI</mark> ICTGG ICCAACGIGC GCICGCIGGA GCGGGGCGICCC ICCGIGAAGI CGCICAICIG	4011	ACCT(4111	GCGTTCCTGG ACAGAGTCGC CGCCACCGAC ACGCTCGACG ACTTCGTGGA CATCGTCGTG GACTATGTAA ATAACTCTGA GCAGGTAAAT GGA <u>TGA</u> GGGG	4211	crececerea ceececeara careaaceae creceecea reaacareae carererer acecaacree acecaacea ecceaacea cerrecea	4311	cectegacea efectecese cassectree resactriea strescesse sesaceiss carescesia ée
1601	-	3701	_	3801	_	3901		4001		4101		4201		4301	_~



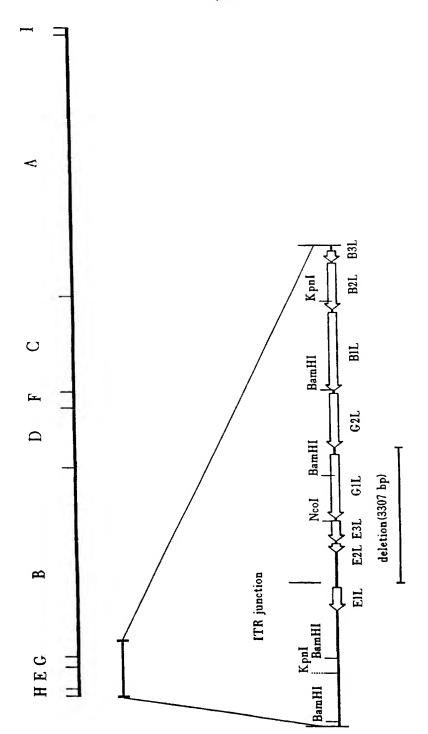


FIGURE 8. BamHI Map of Orf Virus NZ-2 showing the location and orientation of the reading frames for the putative genes E1L(ORF-3), E2L, E3L(ORF-PP), G1L, G2L, B1L, B2L, and B3L.

from orf virus Figure 9. Nucleotide sequence of part of the BamHI E and BamHI G fragments strain NZ-2 showing potential insertion sites.

	-	11 2	21 31	4	1 51		61 7		81 9	91
	 AGTGAGCGGG	CGGGCGATAA	AGTGAGCGGG CGGCGATAA AATAATC <u>AA T</u> TGACTGATT CGCTCGTGAG CGAGCGAAGG GCGGCGGACA AGGCCGCGGG ATGCTGGTCT	<u>rgactgatt</u>	cecrcergag	CGAGCGAAGG	GCGGCGGACA	AGGGCGCGGG	ATGCTGGTCT	AATCTACTAA
101	11	121	1 131	1 141	151	191	171	1 181	191	<u>-</u> -
1	GGCCGATTAC	 AAAAACGGAT	GGCCGATTAC AAAAACGGAT GGGAGACCGG GAGGGAGAGG GTCACACCTC CGAGCGGTGC ATCCGCGCCA GCTGGCGGCG CCACTCGGCC	 Gagggagagg	 GTCACAGCTC	CGAGCGGTGC	ATCCGCGCCA	coracacaca	CCACTCGGCC	ຄວອວວອອວວ
201	1 211	.1 221	1 231	241	1 251	1 261	1 271	1 281	11 291	
	 GCCGCCCAGG	 cccccttgta	GECGECCAGG CEGEGITGIA GECGECEGEC ACGEGGAGGE AGGICAGEIE GAACTECEGGE CEGAGEGEGE GEAGGICGIA GAIGIGEACG GICGEGAEGI	ACCGCGACGC	AGGTCAGCTC	GAACTCCGGC	CCGAGCGCGC	GCACGTCGTA	GATGTGCACG	Greeceacer
301	1 311	1 321	1 331	341	1 351	361	1 371	381	11 391	,-4
	 TCAGCAGCAG	cececerre	TCAGCAGCAG CGCGCCCTTG CGCAGCGAGG CGAAGGTCGC GTGCATGCCG GCGGCGAGCG GGTACACCGG CGAGAGCGTC CCGCCGCCGT GCGCGACCAC	CGAAGGTCGC	GTGCATGCCG	GCGGCGAGCG	GGTACACCGG	CGAGAGCGTC	recececcer	GCGCGACCAC
401	1 411	1 421	1 431	441	1 451	461	1 471	1 481	1 491	
	ceccatetec	 Geoegaage	cecenteres secceptes cecesares taggeres accanaces accentes desarcas seconteres seconteres	CAGGCAGGTC	ACCGAGGCGG	AGCCGTTCGC	GCGGACGGG	cccccrccA	CGAGGGCGGC	CGGCAGCGGC
501	1 511	1 521	i 531	541	551	195	571	581	1 - E1L 591	
	9929229299 	GGCGGAAGAG	GECECEGEGE GECEGAAGAS CCCGCCGAGO AGGAAGCCCA GCGCCACCAG CGCGAGCGCG CCGAGCAGC TGCGCGGCGA GGGGTGCATG CTTGTTGGCT	AGGAAGCCCA	GCCCACCAG	CGCGAGCGCG	CCGAGCAGCC	TGCGCGGCGA	GGGGTGCATG	crrerreecr
601	i 611	1 621	1 631	641	1 651	661	671	1 681	691 691 PEIL	1
	 ccgrctrcg	cercreecee	ecgarerres carcresces eresandece estereses arcceseer ecescesan esteresaer ecraeces certrirene interiores	GGTGTGGGGT	GTCCGCGGCT	GCGGCGGGAG	GGTCTCGACT	GCTAGGCGGT	CCTTTTTCAC	TTTGCTCCGT
701	ITR	7111 7	721	•	741 7	7 127	761 7	771	781	791
	+	CCGGGGCAAG	GGGGCCTGGT CCGGGGCAAG GGCTGCGGCG GGCG <u>CCCGG</u> CGGGCGGGCC GCTACCCCGC GGGCGGCCC GCGGCGCCA GCGCGGGG CAGCCGCCGC	Smail 2000000000000000000000000000000000000)	CTACCCCGC (າ ລວວອວວວວວ	*CGGCCGCC*	ე၅ეეეეეეე	CAGCCGCCGC
801	1 811	1 821	1 831	841	851	861	871	981	1 891	
_	CACCGCGGGC	00000000000	כאכנפכפפס כנפנכפנפנפ באפראפנינים פכנפנפנאפנ פננננפנפנים פננפנפפנים כפנפננפאפנים בפנינפאפנים בפנינפנפנים אפראפנאפנים	CCGCCGAGC	,	ອວອອອວອວວຣ	CGCGCGAGG	22229222	2282822829	AGCAGCAGCG
901	911	1 921	931	941	951	961	971	981	1 991	
_	GCAGCCGCGC	GTCCAGCGGG	פראפרכיפרים מדיכואפרפים ברסכריפרים בלאסכיפרים ברסכרים ברסכרים ברכים ברסכרים בראסרים בראסרים ביסרים ברסכרים ברס	SCAGCGCCGC	GCGCAGCAGC	SCCCCAGCG	SCAGCCGCCC	CGCGCGTCC	930000000	222222222
1001	101	1021	1031	1041	1051	1061	1071	1081	1091	
	CGCCGCCAGC	AGCGCGCGCA	COCCOCCAGC AGCOCOCOCA CCAGCOCCOC COAGOCOCC COCCOCOCAC CAGOTOCTCC ACAACCAGG TGGTGAGCAA GATTCTCGA GAAGTAGGAG	CGAGGGCGCC	3CGCGCGGAC	AGGTGCTCC	ACGAGCAGGG	rggrgagcaa	GGATTCTCGA	GAAGTAGGAG

1111		112	1131	1 1141	1151	1161	1171	1 1181	119	-1-
TCATGTGTGA CGAC	CGAC	AAGGAG	AGACGTTATA	TTAGGCGCGT	CCTACTTCAC	TTTGAAGATG	GTGTAAAGTG	TTAAAACTTG	ACAAGGAG AGACGTTATA TTAGGCGCGT CCTACTTCAC TTTGAAGATG GTGTAAAGTG TTAAAACTTG AACACCGTTC ACTCCACCAC	ACTCCACCAC
1211	!	122	1 123	1 1241	1 1251	1261	1271	1 1281	1291	
 rgccgttacc grgt	- GTGT	ccrecc	CCAAAAGCAA	CCACAGTGCT	TTTTCCACCA	CCTGTTCCAA	ATCCGTTCCA	AAAGCTCCCA	GICCIGCO CCAAAAGCAA CCACAGIGCI TITICCACCA CCIGIICCAA AICCGIICCA AAAGCICCCA ICCAIIGIIG ITAGAACIII	TTAGAACTTT
1311		132	1331	1 1341	1351	1361	1371	1 1381	1391	
TTC TCTA	_£7.	GGTTGT	TTAGTTCCAC	TGCAAGTTTC	GACCATTATC	GITACIGGAC	Arcererree	TAATGAGTTT	CAGAIGITIC ICTAGGINGI ITAGITICCAC TGCAAGITIC GACCATIAIC GITACIGGAC AIGCIGIIGG TAAIGAGIIT AAIAACCAAT CALAAAAAIA	CATAAAAATA
1411		142	1431	1441	1451	1461	1471	1 1481	1491	~
rt Grta	GTTA	TAAAGC	 TANTAAAGTA (GCANACACTT	IAATGTTATA 1	TTTGCCTAA C	CCTCCGTTA	ACACCACCAT	GTIATAATIT GTIATAAAGC TAATAAAGTA GCAAACACIT TAATGTTATA TITTGCCTAA CCCTCGTTA ÁCACCACCA TAACACCACC ÁCTTAAGGTI	ACTTAAGCTT
1511		152	1531		1551	1561	1571	1 1581	11 159	·
CAC CACT	CACT	ACCACC	TCCAACACAC	ATTCTTTTCT	CIAAAGGICC	CCAAATTCCA	CCTCCTGAAC	TTGGACGTTT	THETACEAC CACTACCACC TODACACAC ATTETTITET CTANAGGICC CCANATICCA CCTCCTGAAC TIGGACGIIT TACAGCACCI CCGGGTGIAC	cccccttar
1611		1621	1 1631	1641	1651	← E2L	1671	1 1681	169	
	TTI	GAAGTT	 ccactgrgac	TGTAGATATG	ATACTGTCCT '	TCTCCAGGCA	IGATTAAAGT	GTGTTGTAAT	THECETACCE TITAGAAGIT CCACTGIGAC IGIAGAIAIG ATACIGICCI ICICCAGGCA IGAITAAAGI GIGIIGIAAI TAGIGIIAIC TACGCAACIG	TACGCAACTG
1711	=	1721	1711 1751 1751 1761 1761 1761 1761	1741	1751	1761	1771	1 1781	1 1791	1
TC TCG	50	ATAAA	**************************************	TTTACAATT 1	* PEZL FTGATTAGCT G	ATGTACCAC G	CTGTATCGC	GGCCACCACA	************************************	CCAGTAGAAC
					111111111111	11111111111	187111111111111111111111111111111111111	11:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1		
181		1821	1831	1841	1681	1881	01	•	•	
IGA GTCG	GTCG	_ 	TCAGTGTTGT	CCAAGCAGTT	AACCTCTTGA 7	ACTGCTGGGC)	ACGATATGCG	Tregearatt	CAANTOCAGA GROGOCGOGG TOAGTGTTGT CCAAGGAGTT AACCTCTTGA ACTGCTGGGG ACGATATGCG TTCGCATALT AGCTGAGCTA TCCTGTCTCC	recrerence
1911	=	1921	1931	1941	1951	1961	1971	1 1981	1 199	
CC TCAN	TCAN	AGTCAC	TGTTTCCAAA	GTTANACAGC	ACCACTCCGA (ogrescerce	STAGTCTTCG	TCGATCACGC	CTICITANCE TENNACICAE IGITICENNA GITNANCAGE ACACITECA COTIGECICG GINGICITEG ICGNICACGE CAGEGECEAE GIEGNIANG	GTCGATAAAG
2011		2021	2031	2041	2051	2061	2071	1 2081 1 2081 	2021 2031 2041 2051 2061 2071 2081 2091	
TG CAAG	CAAG	GCCAGA	ACGTGGTGCT	Arecercer	AGCAACCAGA 1	AGGGGGCTTT 1	ATCAGAAGGT	CAGTAAATAC	TGTTTGACTG CAAGGCCAGA ACGTGGTGCT ATGCGTCCGT AGCAACCAGA AGGGGGCTTT ATCAGAAGGT CAGTAAATAC TACGCGACTG CAATGCGAAG	CAATGCGAAG
2111	:	2121	2131	2141	2151	2161	217	2181	2121 2131 2141 2151 2161 2171 2181 2191	
GATGACACA GTCG	Gree	TATGCA	CTACATAGGT O	CTAATCCTGC	GGCACCAGGA C	SATCCTCTGG (TGGTATAGT	GGCGTTTTGG	GGATGACACA GTCGTATGCA CTACATAGGT CTAATCCTGC GGCACCAGGA GATCCTGG CTGGTATAGT GGCGTTTTGG CTGAGGCGAA CAACCTGAAG	CAACCTGAAG
2211		•	←E3L 2231	1 2241	2251	2261	2271	1 2281	1 229	_
_			Ncol		*********			****** PE3L	ADDITION DATEMENT TO SERVICE THE SERVICE TO	SGCGGGTCA
TG TGGC	ည	AGAACT C	AGTITCCGTG TGGCAGAACT CCATGGCTAG GGTGGCGAGC	GTGGCGAGC	GCCGATCGA	TACGGGGGG	ACMITIAN		11111	
11111	-									

Fig 9.2

=-	TGAGGCCGGC 111111111111111111111111111111	CAAAAGGCG	GCGATCTCCT	CGCACAGCAG	CACGCCGCC	AGCCGGCAGC	AGTCCACGAC	SAGCGTGCGC	GCAGCATCA	GCGCTCGAG	111111111	1166CGTCGC
2391	CACGGGCACA : 111111111111111111111111111111111	CCCCCCCTC C	3CGCATGCTT G	AGCTCCTGCA C	3GCGCGCGAG C	2ATGCGCACC	CCACCACGA A	TAGGCCCGT (GCGCCGCG (111111111111111111111111111111	GGTTCGCGC C	GTTCACGCG C	ACAACGACG C
1 2381	CGCCAGGGCG C	GGGAACTCGG C	AGCGCACCGC G	GCGCAGCGCC A	GTCGGCGGCC C	GCTCCGCGCC C	CAGCCCGCGC T	36GTCCACGC C	3GCACGCGTC C	3CAGGCGTTT C	rcrrcccr c	GCTGTCGCA
2371	CGATCTCCGG C	CCCGTAGATG G	TCCGTGTGGC A	cgcccrccc d	GAGCAGCTCC G	ATGTGCTCGA G	GGTCAGGTC C	TACGGCGGCC G	GCGTCCACGA C	cerrecaere e		SAGGAGAGCA G
1 2361	ATGGAGGTCA C	AGAGCGCCGT C	CAGCGTCGCG 7	AGCATGCGCG C	GGGGGGGGG G	CGAGGCCGAC 7	TGTTGCGCA G	CACACGCGTC 1	GCGCCCGTAC G	36CGTCAAGC C	3CAGCGGCGT 1	AGCCAGGAAG
2351	GAAGCACAGG	ATCTTGCCGA A	GCACGTCCAA (GCGCTCCGCC 1	GATGTGATCA G	CGCATAGGCC C	CTGCAGGTTG	ATGGTGTTCC (GCAGCGTTGT (GCCGCCAGC 6	GGTACACGT of 11111111111111111111111111111111111	GCCGCAGAA 7
2341	CGTACGACAG	CATCATGCAG	GACGCGCGCA	CCGCCAGCGC 2741	CCATTGCTCG (TCCGAAACCG (GCAGCGCGGT (GTAGTGCAGC 7	GCGAGGTGGT C	AGCACAGCAG C	TGGTTCTTG G	
1 2331	GCATGCCGC GCGCAAGTCC AGCAGCGAGT CGTACGACAC GAAGCACAGG ATGGAGGTCA CGATCTCCGG CGGCAGGGC CACGGGCACA TGAGGCCGGC CCC AGCAGGCCACA TGAGGCCGGC CACGAAGTCC AGCAGAGAAGTCC AGAGACCGGC AGGACAAGTCC AGAGACCGGC AGAGAGAGACAAGTCC AGAGAAGAAAGAAAAAAAAAA	GATCTGCTCG GCCAGGGAGA GGCGCAGCCG CATCATGCAG ATCTTGCCGA AGAGCGCCGT CCGTAGATG GGGAACTCGG CCGCGCGTC CAAAAAGGCG	TTCTGCACGA AGAGCGCCTT CGCGTCGTCC GACGCGCGCA CCACGTCGCG TCCGTGGGC ÁGCGCACCGC GCGCATGCTT ÒCGATCTCCT TTCTGCACGA AGAGCGCCTT CGCGTCGTCC GACGCGCGCA CCACGTCGCG TCCGTGTGGC ÁGCGCACCGC GCGCATGCTT ÒCGATCTCCT TTCTGCACGA AGAGCGCCTT CGCGTCGTCC GACGCGCGCA CACGTCGCG TCCGTGTGGC ÁGCGCACCGC GCGCATGCTT ÒCGATCTCT TTCTGCACGA AGAGCGCCTT CGCGTCGTCC GACGTCCCA TCCGTCGCG AGCGCACCGC GCGCATGCTT ÒCGATCTCT TCCTTCTT TCCTTCTT TCCTTCTT TCCTTCTT	Genegaaca degaaraaca arcaegaaca eegenegae agaaraagaa eegenegae agaaraaga eegenagaa agaacaagaa egaacaagaa eegaacaagaa agaacaagaa eegaacaagaa	ceceecerce aaccarcaaa Acacaraace cearacarea dararaarea acacacacac daacaacree dreaceece dececeesa cacaceece cacaceece cacacacace cacacaca	TTGTTGCG GCGCAGCATC TCCGAAACCG CGCATAGGCC CGAGGCCGAC ATGTGCTCGA GCTCCGCGCC CATGCGCACC AGCCGGCAGC	GGCGCCGTG GCTGAACACC GCCGCGCGGT GCAGCGCGGT CTGCAGGTTG TTGTTGCGCA GGTTCAGGTC CAGCCCGCGC TCGAGCACGA AGTCCACGAC HILLIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	GCTCCCGT AGGTCGCCAT GTAGTGCAGC ATGGTGTTCC CACACGCGTC TACGGCGGCC GGGTCCACGC CTAGGCCCGT CAGCGTGCCCGT (ACCGTGCCCGT AGGTCCACGC CTAGGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT (ACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGT CACCGTGCCCGTGCCCCGT CACCGTGCCCCGT CACCGTGCCCCGT CACCGTGCCCCGT CACCGTGCCCCGT CACCGTGCCCCGT CACCGTGCCCCCT CACCGTGCCCCCCT CACCGTGCCCCCCT CACCGTGCCCCCCCCCC	GAGATOTT GGCCGTGCGC GCGAGGTGGT GCAGCGTTGT GCGCCCGTAC GCGTCCACGA CGCACGCGTC CGCGCCGGG CGCAGCATCA 11 11 11 11 11 11 11 11 11 11 11 11 11	cggcggag acgccgccg agcacagcag cgccgccagc ggcgrcaag cgrrcaagc ccaggcgrrr gggrrcgcgc cgcgcrcaag	CACGICCT CGCGGATCCA CTGGTTCTTG GCGTACACGT GCACCGGCGT TACGCCGTAG GTGTTGCCCT CGTTCACGCG CGCGCCGCG 1111111111111111111111111	Decedenc Crcgageteg Gegeegregg Gegeegraan Agecaggang Gaggagagen Cettetegesen Gacanceacg Crosestese
1 2321	GCGCAAGTCC	 GCCAGCGAGA 1111111111111111111111111111111111	AGAGCGCCTT	GCGGATCACG	GAGCGTCGGA	CGTTGTTGCG	GCTGAACACC	CAGCTCCCGT	CGGAGATCTT (CGCGGCGGAG 3321	AGCACGTCCT : : : : : : : : : : : : : : : : : :	GCCGCGCGAC
1 2311		 GATCTGCTCG GC :::::::::::	TTCTGCACGA AC	 GCTCGCACGC	 	Gregegeda corterios descadente recsadaces escontagee cangecedae artecteda derecegee carsesace aseesses secosocae in 11111111111111111111111111111111111	~	GCCGCGCTCG CAGCTCCCGT AGGTCGCAT GTAGTGCAGC ATGGTGTTCC CACACGCGTC TACGGCGGCC GGGTCCACGC CTAGGCCCGT CAGCGTGCCC 	ACCATGCCCT CGGAGATCTT GGCCGTGCCC GCGAGGTGGT GCAGCGTTGT GCGCCCGTAC GCGTCCAGA CGCACGGTC CGCGCCGCG CGCAGCATCA ::::::::::::::::::::::::::::::::::	TGTCCACGAG CGCGGCGGAG ACGCGCGGG AGCAGCAGCAG CGCGCCAGG CGTTGCAAGC CGTTGCAGTC GCAGCGGTTT GGGTTCGCGC CGCGCTCGAG :::::::::::::::::::::::::::::::::::	CAGCAGCGG AGCACGTCCT CGGGGATCCA CTGGTTCTTG GCGTACACGT GCAGCGGGGT TACGCCGTAG GTGTTGCCCT CGTTCACGGG CGCGCGGG ::::::::::::::::::::::::::	TECAGEAGEA GEOGEGEGAE CTEGAGETEG GEGEGETEGG GGEGEGAGAA AGCEAGAAG GAGAGAGEA ÉGETGTEGEA GACAACGACG ÉTGGEGTEGE FILITITITITITITITITITITITITITITITITITITI
2301	2401	1 2501	2601	2701	 2801	2901	3001	3101	3201	, 1301) 3401	

	55 :	280	3GT :::		Z :		<u>ا</u> و		
	GCAGGAC	GGCGTG(AGAGCAC		GCGCAA1		AATTGG		
3591	AGACCACGTC CGCGCCCGCC TCCAGCATGA GCGCGACCAC CTCCGGCCGC ACGCGTCGT ACTGCACGATA GGCGTGCAGC GCGTGCGGC CGCAGGAGTC 11111111111111111111111111111111111	CTTGGCTTTC ACGTCCGCAC CGGCCTCCAG CAGCACGCGC ACGATCTCCG CGCACTGCTC GTGCCGCGG AAGTGCACGC AAAGTGAG CGGCGTGCGC CTTGCTTTTTTTTTT	COTOCTOC COCOCAAGTT CACGTCTCCG TCGGTGGCTA CGAGCGCGCG GACCGTTTCG AGGTCCACCT GCCGGACTC CAGGTAGCGG AAGAGCAGGT	1 3891	CCGCGTGCGG GACCACGACG GACTCCCGCG AGACCATGGC GGCGTTTACA AATATTGAAA TCTTTTTCA CTCATCTTTA TGGCGCTGAA CGCGCAATAA	3911 3921 3931 3941 3951 3961 3971 3981 3991	GGGTGAGAGT AANAAACTTC TACAAAAAGC GTACAAAAAGG TACAAAAAGGT AAAAAAGGCG GGGCGGGGAC GGGCTGGGGT GCTGCGAGCT GAATTGGCCT		
1 3581	ACGTA GCCGTGCAGC GCGT 11111111111111111111111111111111111	AAGTGCACGC	GCCCGGACTC	1 3881	CTCATCTTA	1 3981	GGGCTGGGGT		
1 3571	ACTGCACGTA 11111111111	GTGCCGCGCG	AGGTCCACCT	3841 3851 3861 3871 388	TCTTTTTCA	3971	GGCGGGGAC		
1 3561	ACGCCGTCGT A	CGCACTGCTC 111111111111111111111111111111111	GACCGTTTCG	1 386	AATATTGAAA	3961	AAAAAGGCG		
1998	Creegeege A	ACGATCTCCG	CGAGCGCGCG	3851	GGCGTTTACA	3951	TACAAAAGGT		
1 3541	GCGCGACCAC C	CAGCACGCGC	TCGGTGGCTA	3831 ←GlL 3841	AGAGCATGGC	3941	GTACAAAAGG		Ë
1 3531	rccagcarda d	CGGCCTCCAG	CACGTCTGCG	383	GACTCCCGCG	3931	TACAAAAAGC		of deletic
3521	 CGCGCCCGCC	AcgreegeAc	CGCCGAAGTT	3821	GACCACGACG	3921	SEGTGAGAGT AAAAAACTTC TACAAAAAGC GTACAAAAGG TACAAAAGGT AAAAAAGGC GGGCGGGGAC GGGCTGGGGT GCTGCGAGC		endpoint of deletion so Acecerce
3511	AGACCACGTC (ccgractcac		 ccecerecee	3911	GGGTGAGAGT	4011	endpoint cracacaggg accccrccc
3501	3601	3701	_	3801	_	3901		4001	0

Fig 9.4

21/34

FIGURE 10. Orf Virus Transcriptional Promoters.

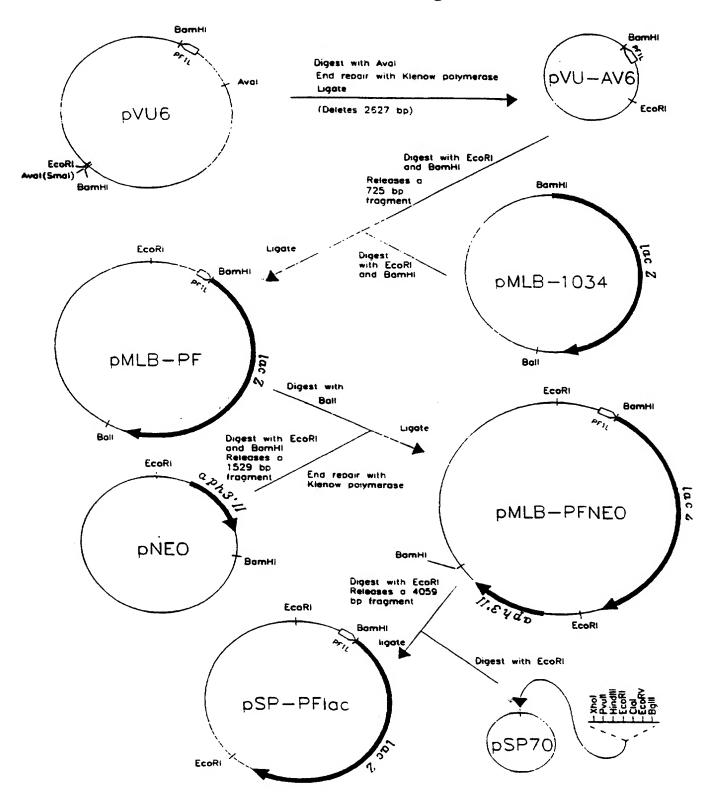
Early Promoters

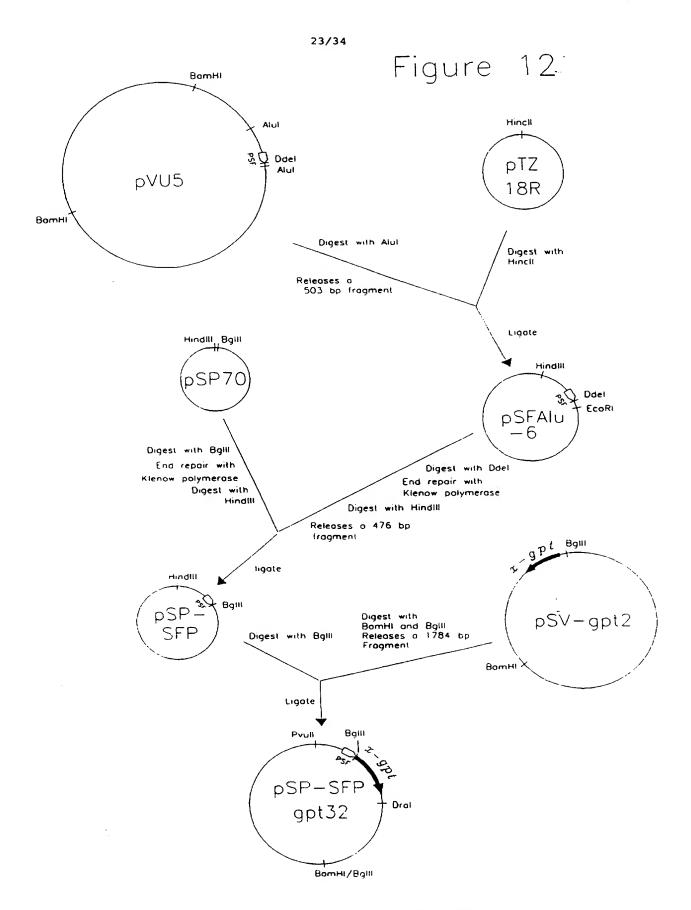
E3L	(ORF-PP)	GAAAGTGTAAATTGTACACCCCGTAGTCGATCGG
E2L	(ORF-1)	AAAATTGTAAAATGTAGCTTCTTTTTATTCGAGA
E1L	(ORF3)	GCAAAGTGAAAAAGGACCGCCTAGCAGTCGAGAC
G1L		GATGAGTGAAAAAAGATTTCAATATTTGTAAACG
G2L		AATAACTGATAAAATATGTTTTTTTTGGTTTTTGGT
BlL		ATAAATTAAAATTAAAGCGCGGAGGCTCGAACGC
B3L		AATTATTGAAAATGTAGGCGCGATAAACACACGT

Late Promoters

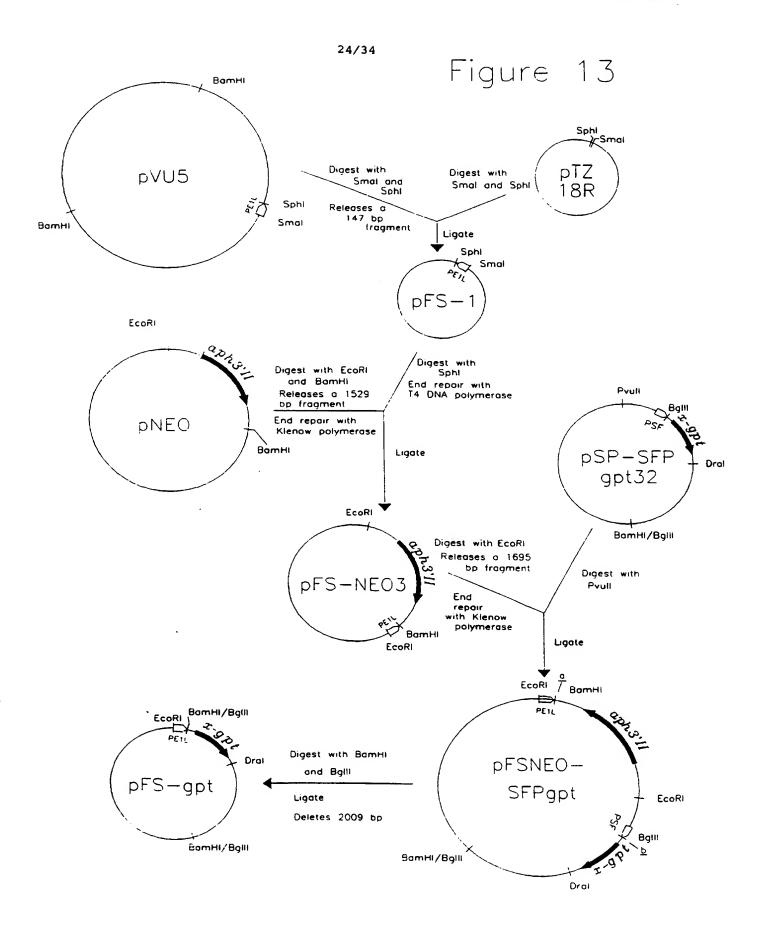
22/34

Figure 11

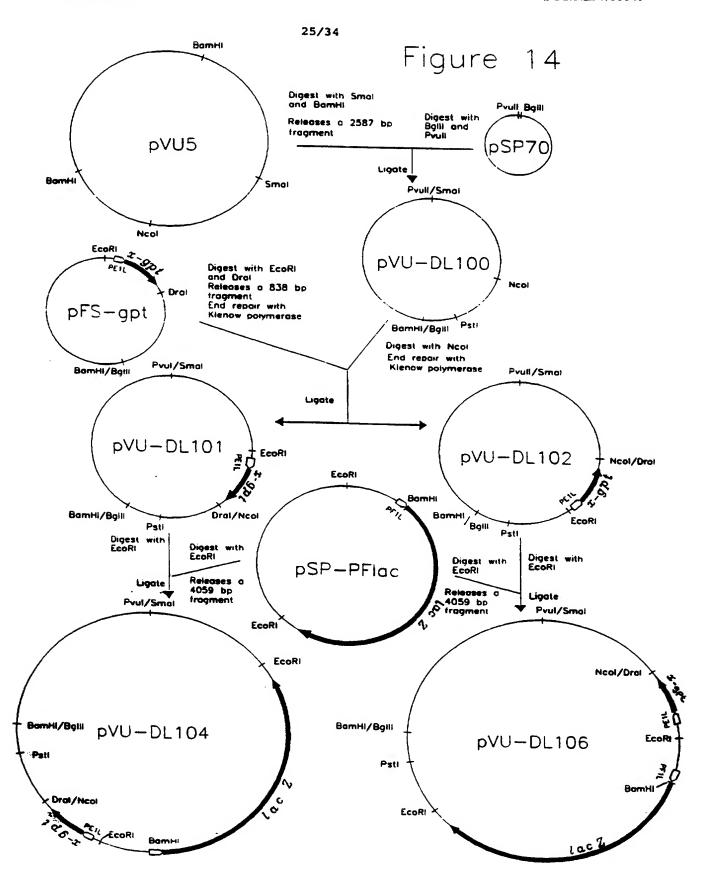




SUBSTITUTE SHEET (RULE 26)



WO 97/37031



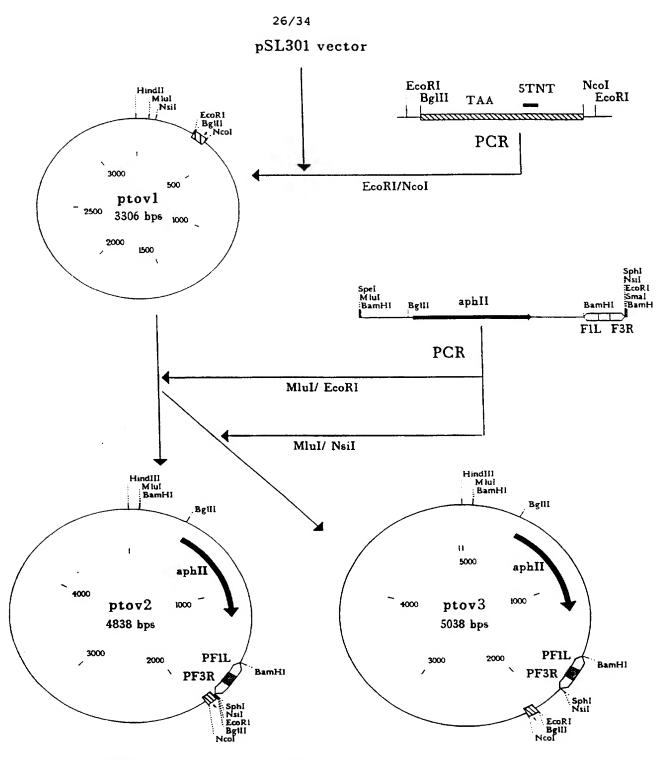


Figure 15. PCR amplification steps involved in the construction of ptov2 and ptov3.

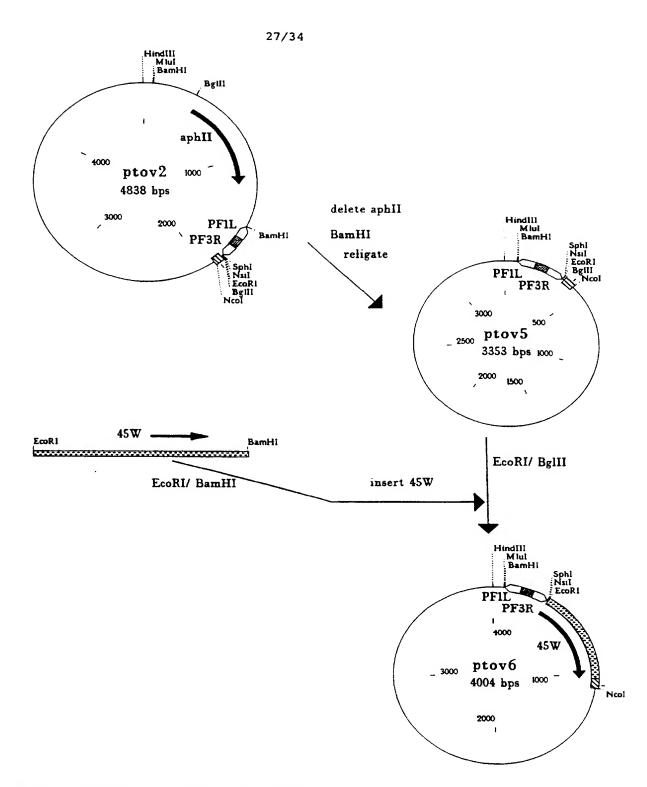


Figure 16. Cloning of the T. ovis 45W antigen into ptov2.

PCT/NZ97/00040

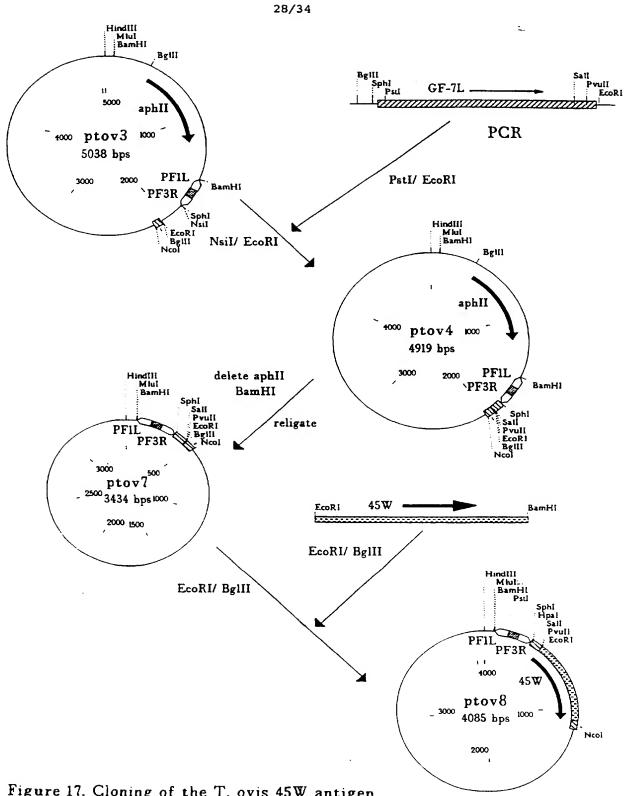


Figure 17. Cloning of the T. ovis 45W antigen into ptov3.

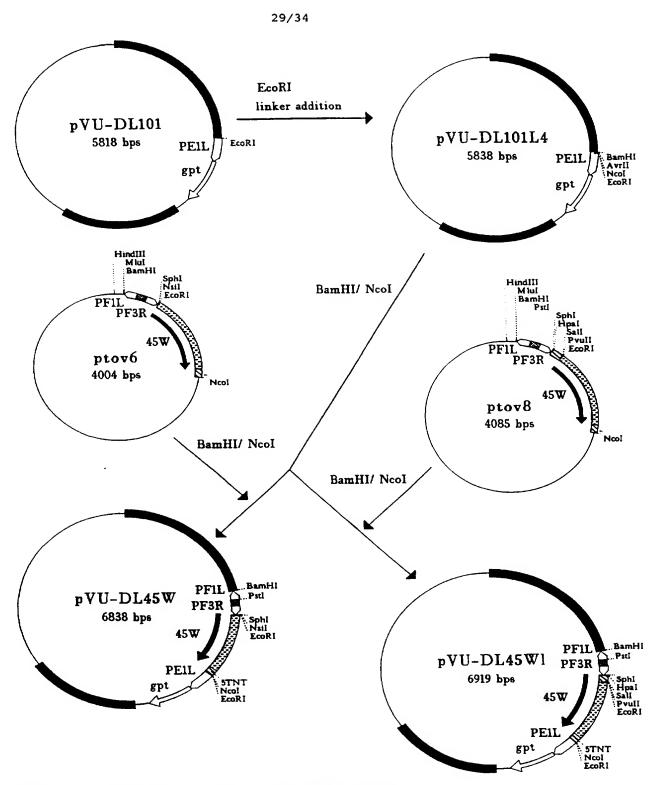
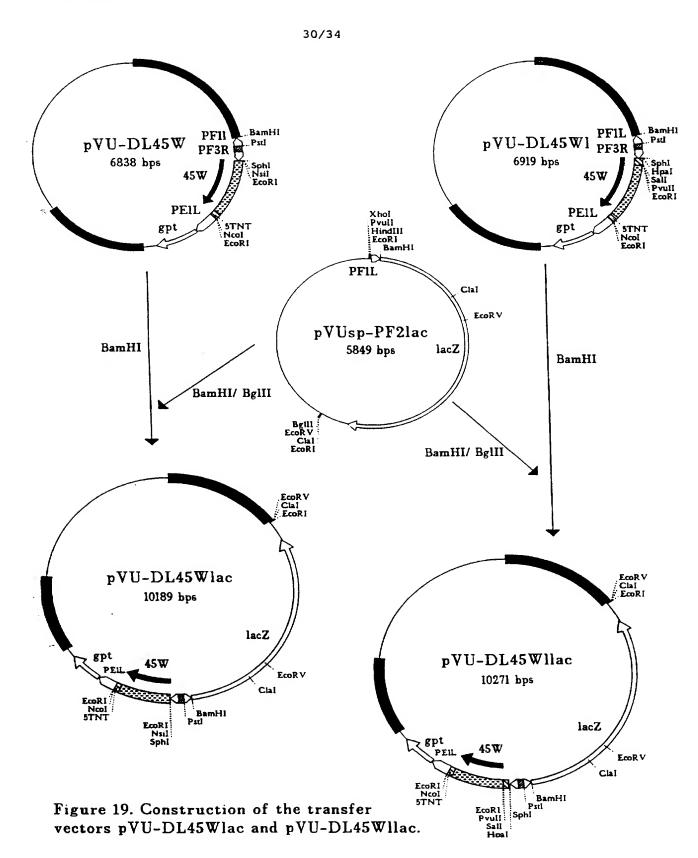


Figure 18. Insertion of the T. ovis 45W antigen into pVU-DL101.



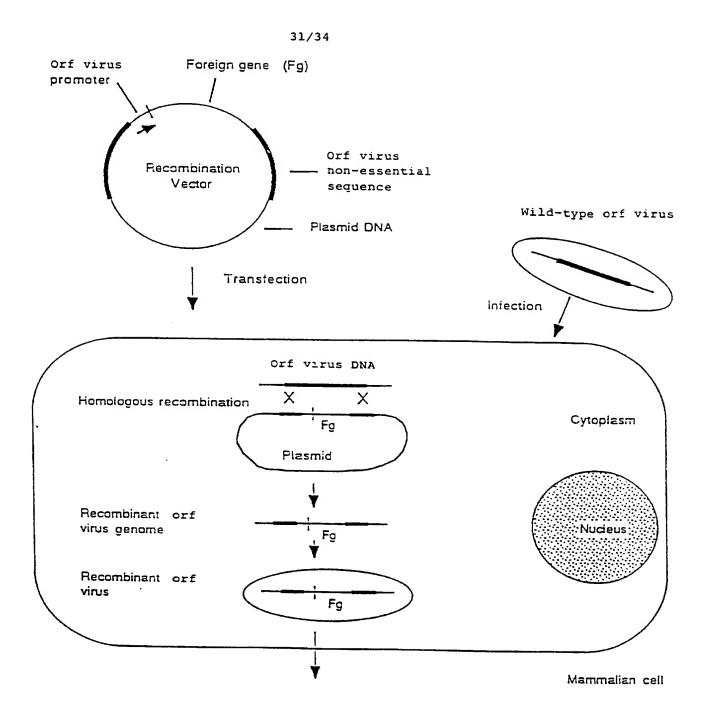


Figure 20. A strategy for the generation of recombinant orf virus.

32/34

zxs-1 GAT CCC G<u>CT CGA G</u>AA CTT CAA

Xhol

zxs-2 GTC <u>AGA TCT ATG CAT</u> AA<u>A AAT TT</u>C GCA TCA GTC GAG ATA

BglII Nsil Apol

zxs-3 GAC <u>ATG CAT</u> CAG TG<u>C CAT GG</u>A ATT CTC GCG ACT TTC TAG C

Nsil Ncol

EcoRI

zxs-4 GAC <u>GGA TCC</u> GTA TAA TGG AAA GAT TC

BamHI

Figure 21A. Primers used for the amplification of orf virus sequences used to create the transfer vectors pTvec1 and pTvec50.

l 11 21 31 41
GACTGATGCG AAACGCGCGG CGGCGCCGCG ACTTAGCTTA TCTCGACTGA

** *********
zxs-2 primer

51 61 71 81 91
TGCGAAATTT TTATGCATCA GTGCCATGGA ATTCTCGCGA CTTTCTAGCT

Apol Nsil Ncol zxs-3 primer

EcoRI

101 TCTCAGACTG ATGCTAC

Figure 21B. Sequence of modified intergenic region between the RNA polymerase subunit gene, rpo 132, and (H)I1L in pTvec50, showing new created restriction sites for the restriction enzymes *Apol*, *Nsil*, *Ncol* and *EcoRI*. The priming sites on the original OV sequence for the zxs-2 primer and zxs-3 primer are markerd by asterisks; the newly created transcriptional termination signal (TTTTTAT) is shown in bold type.

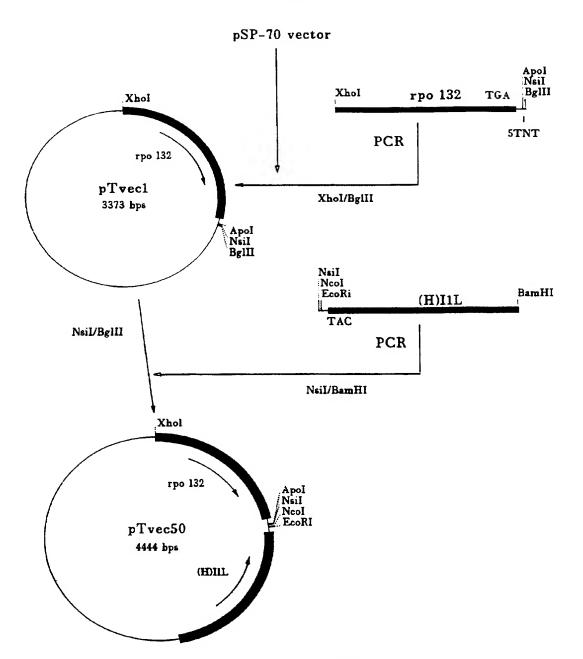
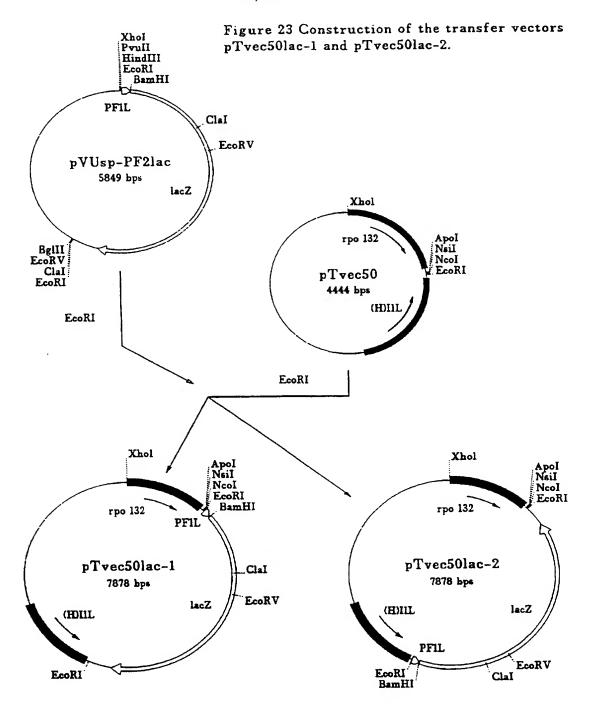


Figure 22 PCR amplification steps involved in the construction of pTvec-1 and pTvec-50.

34/34



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/NZ 97/00040

A.	CLASSIFICATION OF SUBJECT MATTER						
Int Cl ⁶ : Cl	2N 15/86, 5/10; A61K 39/275						
According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED							
B. FIELDS SEARCHED							
Minimum documentation searched (classification system followed by classification symbols) IPC6							
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched SEE DATABASES BELOW							
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPAT - Parapox or orfvirus: or parapoxvirus: or orf () virus: or orfv; CHEMICAL ABSTRACTS - Parapoxvirus; MEDLINE - Parapoxvirus or orf () virus							
C.	DOCUMENTS CONSIDERED TO BE RELEVAN	т					
Category*	Citation of document, with indication, where ap	opropriate. of the relevant passages	Relevant to claim No.				
х	RECOMBINANT POXVIRUSES (1992) Chapt "Parapoxviruses: their biology and potentional Robinson AJ and Lyttle DJ eds M. Binns and G In particular pages 310-316	as recombinant vaccines" by	1-25				
Y	JOURNAL OF GENERAL VIROLOGY (1995) Fleming SB et al. "Genomic analysis of a transpreveals a 3.3 kbp region of non-essential DNA" See entire document.	position-deletion variant of orf virus	1-25				
X	Further documents are listed in the continuation of Box C	See patent family annex					
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document defining the general state of the art which is not considered to be of particular relevance; the claimed invention or document of particular relevance or document of particular relevance or document of p							
Date of the actu 21 May 1997	ual completion of the international search	Date of mailing of the international search	ch report				
	ing address of the ISA/AU	Authorized officer					
	INDUSTRIAL PROPERTY ORGANISATION	Translated officer					
WODEN ACT AUSTRALIA	2606 Facsimile No.: (06) 285 3929	J.H. CHAN					
	(00) 200 0/2/	Telephone No.: (06) 283 2830					

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/NZ 97/00040

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT					
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.			
A	VTROLOGY (1987) vol. 157 pages 13-23 by Robinson AJ et al. "Conservation and variation in orf virus genomes" See entire document, particularly last paragraph.	1-25			